ANNUAL REVIEWS

Annual Review of Vision Science

Genetic and Environmental Risk Factors for Keratoconus

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Annu. Rev. Vis. Sci. 2020. 6:25–46

First published as a Review in Advance on April 22, 2020

The *Annual Review of Vision Science* is online at vision.annualreviews.org

https://doi.org/10.1146/annurev-vision-121219-081723

Keywords

keratoconus, corneal ectasia, genetics, inherited eye disease, complex disease

Abstract

Keratoconus, a progressive corneal ectasia, is a complex disease with both genetic and environmental risk factors. The exact etiology is not known and is likely variable between individuals. Conditions such as hay fever and allergy are associated with increased risk, while diabetes may be protective. Behaviors such as eye rubbing are also implicated, but direct causality has not been proven. Genetics plays a major role in risk for some individuals, with many large pedigrees showing autosomal inheritance patterns. Several genes have been implicated using linkage and follow-up sequencing in these families. Genome-wide association studies for keratoconus and for quantitative traits such as central corneal thickness have identified several genetic loci that contribute to a cumulative risk for keratoconus, even in people without a family history of the disease. Identification of risk genes for keratoconus is improving our understanding of the biology of this complex disease.

INTRODUCTION

Keratoconus [Online Mendelian Inheritance in Man (OMIM) ID 148300] is a complex disease characterized by progressive thinning and a conical protrusion of the cornea. If left untreated, it can result in severe visual impairment or blindness. The etiology of keratoconus is not well understood. Environmental processes such as oxidative stress from UV light–induced damage or eye rubbing are thought to play a role, but there is also a strong genetic component that may provide an underlying susceptibility to such processes in some individuals.

Clinical Signs of Keratoconus

Keratoconus is typically bilateral, although the disease may develop and progress at different rates in each eye. Diagnosis is often made around puberty or during early adulthood, but the disease can develop at any time. Symptoms are subtle in early keratoconus, but as the disease progresses, the cornea thins and protrudes, forming an asymmetric conical shape. Visual acuity is diminished due to high myopia and irregular astigmatism. Clinically, the first conclusive sign of keratoconus is steepening of the cornea (Klyce 2009); however, this is commonly preceded by corneal thinning (Romero-Jimenez et al. 2010). The degree of corneal thinning can vary greatly between individuals, but a meta-analysis of central corneal thickness (CCT) in keratoconus patients showed a mean of 0.434 mm across 12 studies (Doughty & Zaman 2000), significantly lower than population means, which range from 0.513 mm to 0.551 mm (Dimasi et al. 2011). Other early signs include distortion of keratometric images and abnormal light reflexes, such as scissoring of the retinoscopic reflex and oil droplet sign of Charleux (Gordon-Shaag et al. 2015b, Rabinowitz 1998).

As the disease progresses, pigmented iron deposits known as Fleischer's ring are commonly observed around the base of the developing cone (Romero-Jimenez et al. 2010). Fine vertical stress lines, known as Vogt's striae, resulting from the compression of the Descemet's membrane are a sign of moderate keratoconus. Advanced stages are characterized by Munson's sign, which describes the V-shaped deformation of the lower eyelid during downward gaze; an abnormal light reflection known as Rizzuti's sign; scarring of the cornea; and corneal hydrops (Romero-Jimenez et al. 2010). Corneal hydrops is a potentially blinding complication of keratoconus involving breaks in the Descemet's membrane that cause edema of the stroma and loss of corneal transparency. This complication presents with severe pain, corneal opacity, and photophobia and requires immediate corneal transplantation (Maharana et al. 2013, Romero-Jimenez et al. 2010). Vazirani & Basu (2013) reviewed the clinical aspects of keratoconus and provided images of many of the clinical features observed in patients, as well as a summary of current methods for clinical management.

The gold standard technique for diagnosis and monitoring of progression is corneal topography (or videokeratography), a computer-assisted method for mapping and analyzing the corneal surface and curvature (Klyce 1984). This highly sensitive method has allowed for the development of specific indices for the diagnosis of keratoconus (Maeda et al. 1994, Rabinowitz & Rasheed 1999). The most-used indices in research are the central K (CK), describing corneal steepness; the inferior-superior dioptric power asymmetry (IS); and a combination of CK and IS known as KISA. The advantage of measuring topography is that subtle manifestations that do not meet the strict criteria for keratoconus (referred to as forme fruste keratoconus, keratoconus suspect, or subclinical keratoconus) can also be detected (Saad & Gatinel 2010, Ye et al. 2014). Diagnosis and monitoring of subclinical manifestations of the disease are important, as these manifestations may progress and develop into true keratoconus later in life.

Epidemiology

The prevalence of keratoconus ranges between 0.2 (Gorskova & Sevost'ianov 1998) and 4,790 (Torres Netto et al. 2018) per 100,000, and the annual incidence ranges from 1.3 (Nielsen et al. 2007) to 32.3 (Cozma et al. 2005) per 100,000. These statistics vary greatly among studies, methods of diagnosis, geographic locations, and populations, with a notably higher prevalence in Asians and Middle Eastern populations compared to Caucasians. A recent meta-analysis that included more than 50 million individuals from 15 countries determined that the global prevalence of keratoconus was 138 per 100,000 (Hashemi et al. 2019). Some studies report a higher prevalence in men, while others demonstrate a higher frequency in women. When these results are considered together, there is no difference between the sexes, and the conflicting findings might be explained by different age groups included in each study and a tendency for women to develop keratoconus slightly younger than men (Hashemi et al. 2019).

ENVIRONMENTAL RISK FACTORS

The environmental triggers for keratoconus remain largely elusive, although progress has been made on identifying risk factors. Many of the studies evaluating risk factors are relatively small, with fewer than 500 cases. Several larger studies, mostly using medical or billing records to assess the association of a broad range of potential risk factors, have emerged in recent years (**Table 1**).

Allergy and Asthma

Multiple small studies have suggested links between allergy, atopy, or asthma and keratoconus; however, the most convincing studies are those on larger numbers of individuals. The largest

Table 1 Studies of more than 500 keratoconus patients assessing risk factors for keratoconus

Reference	Country	Data source	N cases	N controls	Matched?	Allergy	Asthma	Diabetes
Seiler et al. (2000)	Germany	Notes	571	571	Yes	NA	NA	–
Kuo et al. (2006)	United States	Code	2,102	232,548	No	NA	NA	–
Kosker et al. (2014)	United States	Notes	1,377	4,131	Yes	NA	NA	+
Naderan et al. (2014)	Iran	Notes	1,383	1,383	Yes	NA	NA	–
Merdler et al. (2015)	Israel	Code	807	>600,000	No	+	+	NA
Woodward et al. (2016)	United States	Code	16,053	16,053	Yes	+[a]	+	–
Naderan et al. (2017)	Iran	E + Q	885	1,526	No	+	+	NA
Bak-Nielsen et al. (2018)	Denmark	Code	2,679	26,790	Yes	+	+	No association

[a]Significant in univariate but not multivariate analysis.

The column on matching indicates whether the case and control group were matched for relevant demographics. Notes refers to a review of medical records; Code refers to data extracted from coded billing or claims records; E + Q indicates examination and questionnaire; + indicates a risk for keratoconus; – indicates that a factor is protective against keratoconus.

Abbreviation: NA, not assessed.

study to date (Woodward et al. 2016) utilized coded billing records from a managed care network in the United States to identify 16,053 keratoconus patients and matched controls with similar demographics to each case. A similar study from Israel (Merdler et al. 2015) used coded records obtained during medical assessments of teenagers during military service. They compared 807 cases to over 600,000 controls. Another study from Denmark (Bak-Nielsen et al. 2018) investigated 2,679 keratoconus patients and matched controls identified from several nationwide civil and medical registers. As shown in **Table 1**, these three large studies all report both allergy and asthma as risk factors for keratoconus (Bak-Nielsen et al. 2018, Merdler et al. 2015, Woodward et al. 2016). The study in Denmark (Bak-Nielsen et al. 2018) also found an association with atopic disease, which was not assessed by the other studies. The challenge in interpreting these studies is understanding the accuracy and scope of the codes used to record a diagnosis of keratoconus and the myriad of potential risk factors. These can differ markedly between studies conducted in different countries or within different health systems. The biases and noise are somewhat overcome by the large sample sizes that are often not possible in studies examining individual patients. One can also have confidence in the studies due to their general agreement on the direction of the association, even with different outcome and exposure definitions and different populations. The largest study to directly assess individual patients for keratoconus risk factors was conducted in Iran (Naderan et al. 2017). The authors recruited 885 participants with keratoconus, a number comparable to the medical record review in Israel (Merdler et al. 2015). They reported a similar association of both allergy and asthma with keratoconus. Several other smaller studies using detailed review of medical notes or direct patient examination and questionnaires also concluded that allergy and asthma are risk factors for keratoconus (Naderan et al. 2015, Nemet et al. 2010, Shneor et al. 2013).

Eye Rubbing

It has long been hypothesized that the mechanical trauma induced by prolonged or aggressive eye rubbing may lead to keratoconus, and several plausible mechanisms have been suggested (McMonnies 2009). There have been numerous studies evaluating the link, primarily in small cohorts of under 100 patients. The methods for measuring eye rubbing have not been standardized, although there has been a recent trend of using visual analog scales. Studies consistently report significant association of the frequency or intensity of eye rubbing with keratoconus (Bawazeer et al. 2000; Gordon-Shaag et al. 2013, 2015a; McMonnies & Boneham 2003; Naderan et al. 2015; Weed et al. 2008). It is strongly hypothesized that allergy and atopy lead to increased eye rubbing, and thus that these allergic diseases are the root cause. Four small studies that evaluated both allergy or atopy and eye rubbing support this view (Bawazeer et al. 2000, Gordon-Shaag et al. 2013, McMonnies & Boneham 2003, Naderan et al. 2015); however, none of these studies were adequately powered for multivariate analysis to evaluate the independence of these risk factors. None of the large studies with allergy data (Bak-Nielsen et al. 2018, Merdler et al. 2015, Naderan et al. 2017, Woodward et al. 2016) have evaluated eye rubbing due to their reliance on coded records. Shneor et al. (2013) documented eye rubbing and allergy rates across 16 studies and reported that the rate of allergy is in the range of 30–40% of keratoconus patients, but that eye rubbing is reported by 50–70% of patients. They suggest that, although allergy might play a role, it does not account for all eye rubbing. This observation, combined with the data suggesting that not all keratoconus patients rub their eyes, highlights the heterogeneity of keratoconus.

Diabetes

The association of diabetes with keratoconus was first evaluated by Seiler et al. (2000). They developed the hypothesis that diabetes would be protective against keratoconus by enhancing

nonenzymatic glycosylation, which increases protein cross-linking, as seen in other tissues of diabetic patients. To test this, they undertook a retrospective study of medical records of patients between 20 and 40 years old seen in the ophthalmology departments of three German universities. They found a significantly lower rate of diabetes in the 571 eligible keratoconus patients than in age- and sex-matched patients attending the same clinics for a range of other (noncorneal and nondiabetic) ophthalmic indications. Two other studies with similar methodology replicated these findings (Kuo et al. 2006, Naderan et al. 2014), and one also reported an association between diabetes and a reduced severity of keratoconus, defined by visual acuity (Kuo et al. 2006). In contrast, the study of Kosker et al. (2014) found a higher rate of type 2 diabetes among keratoconus patients than controls. They also showed that diabetes was associated with more severe keratoconus, defined by K-readings. The reason for this opposite effect is not clear, but biases created through clinic-based case-control recruitment, the relatively small sample size in all four studies, the temporal relationship between diabetes and keratoconus, and a failure to account for the degree of control of diabetes in any of the studies may be important factors. As with the assessment of allergy, the best-powered study to date to evaluate the association between diabetes and keratoconus is that of Woodward et al. (2016), who show a clear protective effect of diabetes in their cohort of over 16,000 patients. They showed an even stronger effect of complicated diabetes (a proxy for poorer diabetic control or longer duration of diabetes), but they were also unable to adjust specifically for diabetes duration and control. The power of this large cohort study provides reasonable confidence in the findings of a protective effect of diabetes in keratoconus; however, under multivariate analysis adjusting for all of the measured risk factors, the effect of diabetes is borderline. Given that the onset of type 2 diabetes is typically much later than the onset of keratoconus, the clinical relevance of this association is not immediately evident. The role of glycosylated proteins in promoting collagen cross-linking to stabilize early keratoconus is an area of potential future research.

EVIDENCE FOR A GENETIC COMPONENT TO KERATOCONUS

Coinheritance of Keratoconus with Genetic Diseases

Keratoconus has been reported in association with a long list of genetic conditions (Rabinowitz 2003), although it is highly likely that some case reports of comorbidities are coincidental. Establishing firm links with rare diseases is challenging; however, several interesting associations with genetic diseases have been reported. A recent study in Down syndrome patients from Spain and Egypt found that up to 75% of Down syndrome patients have ocular features consistent with keratoconus (Alio et al. 2018). This rate is higher than reported in most previous studies but is attributed to the use of modern corneal topography to identify keratoconic features not available to earlier studies. A similar sixfold increased rate of Down syndrome in keratoconus patients compared to controls was reported in the study of Woodward et al. (2016) from the United States, further strengthening the evidence for an association.

Keratoconus is reportedly common in families with the rare genetic disease Leber congenital amaurosis (LCA). This heterogeneous disease is characterized by a distinctive retinal dystrophy with at least 16 genes known. Keratoconus may be a feature associated with some LCA-causing genetic mutations (Dharmaraj et al. 2004, McMahon et al. 2009); for other mutations, the genetic link is less clear. For example, an LCA-causing mutation was identified in a consanguineous family with four children affected by both LCA and keratoconus (Hameed et al. 2000) but was also reported in two unrelated families with isolated LCA, suggesting that it was not the cause of the keratoconus in the original family (Sohocki et al. 2000). Further study of individuals with both LCA and keratoconus is needed to shed light on whether genetic or environmental factors are the primary causes of keratoconus in these patients.

There are many reports of co-occurrence with connective tissue disorders such as Ehlers-Danlos syndrome, Marfan syndrome, and osteogenesis imperfecta (Rabinowitz 1998). Mitral valve prolapse is part of these collagen disorders, and an association between keratoconus and mitral valve prolapse is also reported (Kalkan Akcay et al. 2014, Rabinowitz 1998). Mitral valve prolapse is one of the most common genetic conditions known, with a frequency of 2–3% of the adult population; however, the genetics are not well understood (Le Tourneau et al. 2018). Some families have clearly inherited high-penetrance mutations (Durst et al. 2015), but the disease is heterogeneous in inheritance pattern and genetic risk factors, as is keratoconus. Co-occurrence may ultimately be attributable to the prevalence of both conditions.

Familial Clustering of Keratoconus

Evidence of a genetic component for keratoconus comes from studies evaluating family history of the disease. The proportion of keratoconus patients who report a family history of the disease ranges from 5% in Scotland (Weed et al. 2008) to 28% in Israel (Shneor et al. 2013), with most studies falling between 10% and 25% (Bawazeer et al. 2000; Gordon-Shaag et al. 2013, 2015a; Ihalainen 1986; Naderan et al. 2015; Owens & Gamble 2003; Zadnik et al. 1998). This is a much higher rate than that seen in matched controls in the few studies that did recruit and evaluate controls (Bawazeer et al. 2000; Gordon-Shaag et al. 2013, 2015a; Naderan et al. 2015). A comprehensive study of a large series of keratoconus cases and their nuclear families found a prevalence of 3.34% in first-degree relatives (Wang et al. 2000), which was at least 15-fold higher than the reported prevalence in that population. A similar study found an even higher rate of 7.6% (Kriszt et al. 2014). Both studies evaluated family members who had not been previously diagnosed with keratoconus. Although most keratoconus appears to be sporadic (notwithstanding undiagnosed relatives), there are numerous reports of extended pedigrees with apparent autosomal-dominant or autosomal-recessive keratoconus, indicating Mendelian inheritance and the likely presence of major gene effects in those families (Brancati et al. 2004, Burdon et al. 2008, Bykhovskaya et al. 2014, Gajecka et al. 2009, Hughes et al. 2003, Nowak et al. 2013, Tang et al. 2005). Analysis of quantitative traits derived from videokeratography also provides evidence of the heritability of keratoconus. Wang et al. (2000) reported significant familial correlations of CK, IS, and KISA, even including family members who were not clinically affected with keratoconus. Using segregation analysis of KISA data in 95 families with at least three affected individuals, their study also suggested that a recessive gene with large effect may be responsible for keratoconus, although polygenic and environmental contributions were not ruled out. Similarly, Kriszt et al. (2014) reported familial correlations of KISA and suggested a major gene effect with varied inheritance patterns. As discussed below, research to date has not identified a single major gene, although in some families the inheritance pattern does suggest such a model. It is clear that keratoconus is a complex disease, and while some families present with obvious Mendelian inheritance, multiple factors are at play in most individuals.

FAMILY-BASED STUDIES FOR GENE IDENTIFICATION

Linkage Studies

Linkage studies have highlighted the genetic heterogeneity of keratoconus, with suggestive and significant linkage regions identified throughout the genome (**Figure 1**). Although linkage studies involving multiple small families have the potential to miss key loci due to heterogeneity, these methods have identified six loci significantly linked to keratoconus—5q32–33 (Bisceglia et al. 2009), two regions on chromosome 9 (Li et al. 2006), 14q24.3 (Liskova et al. 2010), 16q22.3–23.1

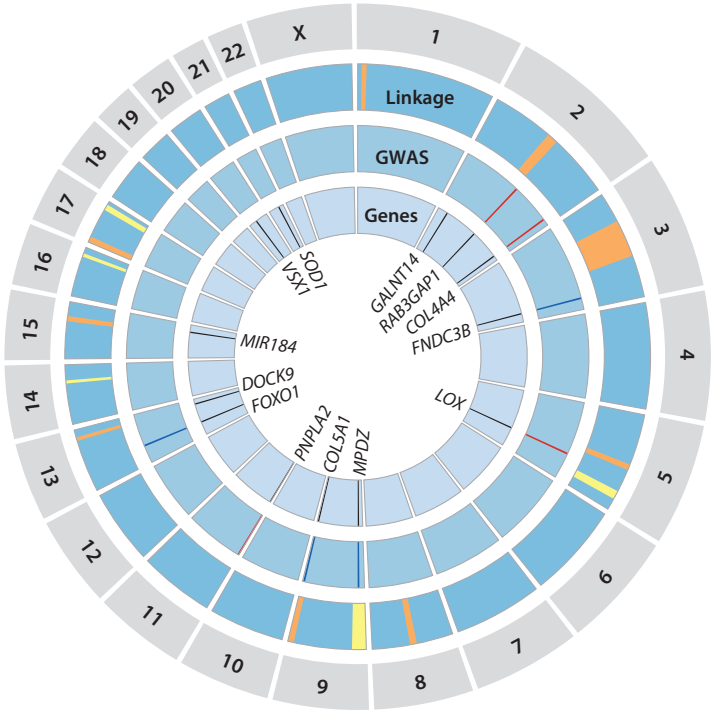

Figure 1

A circos diagram depicting keratoconus-associated loci and the location of candidate genes discussed in this review. The grey outer track displays the reference genome (hg19), with chromosomes depicted by grey blocks relative to their size. The linkage track displays the location of linkage regions identified using extended pedigrees (*orange*) and multiple small families (*yellow*). The genome-wide association study (GWAS) track displays the location of single nucleotide polymorphisms associated with keratoconus identified through GWASs for keratoconus (*red*) and central corneal thickness (*dark blue*). The genes track displays the location of candidate genes (*black*), with gene names located in the center of the plot.

(Tyynismaa et al. 2002), and a region on chromosome 17 (Li et al. 2006)—as well as many regions suggestive of linkage (Bisceglia et al. 2009, Li et al. 2006, Liskova et al. 2010). Linkage analyses in six extended pedigrees with strong Mendelian inheritance patterns have identified an additional six loci with at least suggestive linkage with keratoconus: 2q13-q14.3 (Nowak et al. 2013), 3p14-q13 (Brancati et al. 2004), 5q15-q21.1 (Bykhovskaya et al. 2014, Tang et al. 2005), 13q32 (Czugala et al. 2012, Gajecka et al. 2009, Karolak et al. 2015), 15q22.33-q24.2 (Hughes et al. 2003, 2011), and 17p13 (Hameed et al. 2000). In addition, the analysis of a large Australian family with apparent autosomal-dominant keratoconus revealed strong evidence that keratoconus was inherited as a digenic trait and identified linkage regions on two different chromosomes with equal evidence of linkage: 1p36.23–36.21 and 8q13.1-q21.11 (Burdon et al. 2008). While it is possible that one or both of these linkage regions may have cosegregated with keratoconus by chance, this finding highlights the complex nature of keratoconus genetics, even in families with apparently Mendelian inheritance patterns of disease.

Genes Identified from Family Studies

Linkage analysis in families highlights the regions of the genome that are most likely to contain a causative variant in the family. Further detailed analysis of the region(s) in affected and

unaffected family members aims to pinpoint the specific mutation responsible for the disease. This is not necessarily straightforward, but several examples exist of successful gene identification for keratoconus in families.

MIR184. One of the first large families with keratoconus to be described was a three-generation Northern Irish family with cosegregating keratoconus and pediatric cataract with autosomal-dominant inheritance (Hughes et al. 2003) showing linkage to a 5.5 Mb region on 15q22.33–24.2 (Dash et al. 2006). Via targeted resequencing, a novel heterozygous variant (r.57c>u) in *MIR184* was proposed as the causative variant (Hughes et al. 2011). *MIR184* encodes miR-184, the most abundant microRNA in corneal and lens epithelia (Ryan et al. 2006). The r.57c>u variant is located in the seed region of miR-184, the region that binds to complementary target messenger RNA (mRNA) molecules. (Hughes et al. 2011). Using a cell model system, the authors showed that this variant influenced expression of target genes through altered competitive binding with miR-205. This work confirmed that the *MIR184* r.57c>u variant had a functional effect on protein expression; however, the critical target genes in the cornea and altered interactions that cause the phenotype observed in the family have not yet been elucidated.

The same *MIR184* r.57c>u variant was identified in a Caucasian-American family with a broader ocular phenotype with linkage to 15q22.1–25.3 (Iliff et al. 2012, Jun et al. 2002). The phenotype included endothelial dystrophy, iris hypoplasia, congenital cataract, and stromal thinning, dubbed EDICT syndrome (Akpek et al. 2002). It was also found to segregate in a Spanish family with a combined cataract and corneal phenotype (Bykhovskaya et al. 2015a). The phenotype observed in the Spanish family was reminiscent of the Northern Irish family, as neither family displayed iris defects. Only the proband in the Spanish family was diagnosed with keratoconus, and all other affected family members displayed uniform corneal thinning (Bykhovskaya et al. 2015a). Despite the phenotypic similarities, the authors demonstrated that the two families did not share a common haplotype, indicating that the *MIR184* r.57c>u variant was a recurrent germline variant (Bykhovskaya et al. 2015b). They went on to hypothesize that the differences in phenotype, both within and between families, may be modulated by polymorphisms located within the gene targets of miR-184 (Bykhovskaya et al. 2015b). There is also a chance that the keratoconus was inherited independently of the *MIR184* r.57c>u variant in the Spanish family, as the paternal grandmother also had keratoconus, while the *MIR184* r.57c>u variant was maternally inherited.

MIR184 has now been screened in four ethnically diverse keratoconus cohorts, as well as a small Chinese cohort with axial myopia (Abu-Amero et al. 2015b, Cagil et al. 2017, Farzadfard et al. 2016). A total of 1,144 unrelated keratoconus patients were included across all four studies; however, only two potentially pathogenic variants were identified: miR-184(+3A>G) and miR-184(+8C>A) (Lechner et al. 2013). Two Caucasian patients were identified, each carrying one of these variants, and neither displayed any evidence of anterior segment dysgenesis or iris abnormalities. Furthermore, the patient with miR-184(+8C>A) had no lens abnormalities. The patient with miR-184(+3A>G) had both keratoconus and cortical granular cataracts, as did his brother, who also carried the same variant. Their mother had similar cataracts, but the miR-184(+3A>G) variant was paternally inherited. This suggests that the cataracts and keratoconus were inherited independently, assuming that the miR-184 variant is responsible for keratoconus (Lechner et al. 2013). Both variants are located in the stem and loop region of the precursor miR-184 and were predicted to alter the stability of the secondary structure using in silico tools. An ex vivo model demonstrated that both variants significantly reduced the expression of the mature miR-184 compared to the wild-type precursor (Lechner et al. 2013). Taken together, this evidence indicates that functional variants in *MIR184* are rare causes of anterior segment dysgenesis, including keratoconus, and may contribute to isolated keratoconus in a small number of patients.

LOX. A large linkage study including 351 individuals across 67 small families identified regions with at least suggestive linkage (logarithm of the odds score >2) on nine chromosomes, including a peak at 5q23.2 (Li et al. 2006). This peak was of particular interest because it encompassed the *lysyl oxidase* (*LOX*) gene, which had previously been shown to be differentially expressed in corneal epithelia from keratoconus patients compared to healthy controls (Nielsen et al. 2003). The *LOX* gene encodes a copper-dependent extracellular enzyme that cross-links collagens and elastin in a variety of tissues, including the cornea. As both a positional and biological candidate gene for keratoconus, *LOX* was screened by direct sequencing in an Italian cohort of 302 keratoconus patients (De Bonis et al. 2011). Known polymorphisms were identified but none were considered to be disease causing. Subsequently, Bykhovskaya et al. (2012) conducted association analyses for variants in *LOX* in two independent Caucasian case-control cohorts and a familial cohort consisting of Caucasian and Hispanic individuals. A meta-analysis of these results revealed that two intronic single-nucleotide polymorphisms (SNPs) were associated: rs10519694 ($p = 4.0 \times 10^{-5}$) and rs2956540 ($p = 2.5 \times 10^{-7}$). The association at rs2956540 was replicated in a Han Chinese cohort (Hao et al. 2015) and a Czech cohort (Dudakova et al. 2015). In contrast, rs10519694 did not replicate (Dudakova et al. 2015). When considering only the case-control cohorts, rs10519694 showed a nominal association ($p = 0.03$), while rs2956540 reached genome-wide significance ($p = 1.4 \times 10^{-8}$) (Zhang et al. 2015). To date, there are no functional data to support a direct link between any particular variant in the *LOX* gene and keratoconus pathogenesis. The association studies do suggest a role for the locus, indicating that it is worthy of further evaluation.

DOCK9. An Ecuadorian family with 10 affected family members across three generations showed significant linkage to a 5.6 Mb region on 13q32 (Gajecka et al. 2009). Eight of the 25 genes in the region were selected as candidates and screened using direct sequencing methods (Czugala et al. 2012). Only one variant, c.2262A>C in the *dedicator of cytokinesis 9* (*DOCK9*) gene, was shared by all affected family members and was absent in 105 ethnically matched controls (Czugala et al. 2012). The *DOCK9* gene encodes a guanine nucleotide–exchange factor that activates a GTPase involved in the regulation of cell adhesion and migration (Kulkarni et al. 2011). The DOCK9 protein contains three key domains, a pleckstrin homology (PH) domain and two evolutionarily conserved dock homology regions (DHRs), DHR1 and DHR2. The PH and DHR1 domains mediate membrane localization, while the DHR2 domain is the catalytic domain responsible for the guanine nucleotide-exchange activity (Cote & Vuori 2006, Cote et al. 2005, Meller et al. 2002).

The c.2262A>C variant occurs at the penultimate nucleotide in exon 20 of *DOCK9* and is predicted to result in a nonsynonymous substitution within the DHR1 domain, p.(Q754H), and to impact gene splicing (Karolak et al. 2015). When HeLa cells expressing mini gene constructs containing the variant were used, an aberrant transcript lacking exon 20 was observed, along with a low level of the wild-type transcript, confirming that the variant could induce exon skipping in vitro (Karolak et al. 2015). The aberrant transcript was predicted to produce a premature stop codon, resulting in a truncated protein with only 722 amino acids, disrupting the DHR1 and eliminating the DHR2 domain (Karolak et al. 2015). Further investigation could not confirm the presence of the aberrant transcript in RNA samples extracted from lymphoblastoid cell lines derived from affected family members; however, Karolak et al. (2015) presented evidence that the aberrant transcript may undergo nonsense mediated decay. While further evidence is required to confirm the role of the c.2262A>C variant and *DOCK9* in keratoconus, this work highlights one of very few candidate variants or genes with functional support.

Massively parallel sequencing methods and *GALNT14*. In recent years, genetic studies have often bypassed linkage analysis and have instead utilized whole-exome sequencing or whole

genome sequencing to identify candidate variants that segregate with disease in families without a priori hypotheses. Massively parallel sequencing methods can also be applied in combination with linkage analysis to focus analyses on smaller regions of the genome and fast-track the identification of causative variants while removing the biases and time-limiting step of sequencing individual candidate genes.

A promising candidate gene identified using this technology is *GALNT14*. Via exome sequencing data, a novel, homozygous, loss-of-function variant (c.60delC) was identified in the two affected children of unaffected, first-cousin parents from Jordan (Froukh & Hawwari 2019). The variant is predicted to result in a truncated protein only 27 amino acids in length [p.(L21Cfs*6)]. To date, no homozygous loss of function variants in *GALNT14* have been observed in the large publicly available data set of human genome sequences in gnomAD (Lek et al. 2016). The *GALNT14* gene encodes a glycosyltransferase that catalyzes the first step in the O-glycosylation of target proteins, including mucins. Ocular mucins are glycoproteins that play a critical role in the protection of the epithelia in both the cornea and conjunctiva. In healthy corneas, 55% of the structure of ocular mucins is made up of carbohydrates attached to the protein core (Chao et al. 1988), corresponding to O-linked glycans. Alterations in ocular mucins have been described in ocular allergy, dry eye, and infectious diseases of the ocular surface (for a review, see Mantelli & Argueso 2008). Current therapies for keratoconus (reviewed elsewhere) aim to increase cross-linking of proteins, a process that is enhanced by glycosylation, as seen through increased cross-linking in tissues of patients with diabetes (Seiler et al. 2000). The *GALNT14* gene is a strong candidate for keratoconus but requires further assessment of both the specific variant and the gene itself in large, unbiased case-control studies to confirm a role in disease, as well as carefully designed functional studies to determine the mechanism of disease.

CASE-CONTROL AND COHORT STUDIES

Candidate Genes

Candidate genes are often proposed based on a gene's function, expression pattern, or proximity to an association signal or linkage peak (**Figure 1**). Despite dozens of genes having been hypothesized to play a role in keratoconus susceptibility, the collective results of candidate gene studies in keratoconus have not led to substantial success. Most studies screened candidate genes to identify variants present in cases and absent in controls or with significantly different allele frequencies.

Many published candidate gene studies have small numbers of patients and/or controls ($n < 100$). Small sample sizes do not allow the frequency of rare variants to be accurately estimated and can lead to false conclusions. Many studies also did not fully screen the controls for rare variants and instead only assessed control samples for the variants that were identified in the case cohort. This led to a lack of recognition of the true variability of several key genes in the general population. These issues may have stemmed from a lack of appreciation in the genetics community of the vast number of benign rare missense variants, even in healthy individuals. Furthermore, few follow-up studies have been conducted to determine if the specific variants identified actually have a role in keratoconus susceptibility or are biologically relevant. Some papers have been able to demonstrate a functional effect for some variants; however, none have gone as far as linking the functional effect to a disease mechanism. Together, these issues have limited the success of candidate gene studies in keratoconus and perpetuated the field's focus on candidate genes with limited evidence. The *visual system homeobox 1* (*VSX1*) gene is a prime example of this cautionary tale.

VSX1. In the landmark paper by Heon et al. (2002), the transcription factor *VSX1* was reported as the first gene for keratoconus. *VSX1* was initially proposed as a candidate gene for posterior

polymorphous corneal dystrophy (PPCD; OMIM ID 122000). The gene was located within a linkage region identified in a family with 21 PPCD cases and was known to be expressed in ocular tissues. The authors were unable to confirm *VSX1* expression in adult cornea, and no variants were identified in the PPCD family; however, they explored *VSX1* in keratoconus due to the co-occurrence of PPCD and keratoconus in a small number of case reports (Heon et al. 2002). In their cohort of 63 keratoconus patients, four missense variants were identified, two of which were absent in controls. Based on these findings, the authors concluded that missense variants within *VSX1* were the cause of keratoconus in at least 4.7% of cases. This finding was supported by Bisceglia et al. (2005) in their subsequent paper, which identified missense variants in *VSX1* in seven out of 80 (8.8%) Italian keratoconus patients. The authors suggested that two previously reported variants, p.(G160D) and p.(D144E), were likely to be rare polymorphisms based on their observations in unaffected relatives and controls. They also suggested that a variant that was previously observed in a healthy married-in spouse in a PPCD family was likely to cause keratoconus in one of their families with keratoconus [p.(P247R)]. This is controversial, as the spouse from the PPCD family did not have keratoconus and is an example of an unaffected control carrying the variant of interest. The alternative interpretation is that this variant is not associated with PPCD or keratoconus.

Aldave et al. (2005) published the first paper that did not support a role for *VSX1* in keratoconus. This study screened the five known exons and intron–exon boundaries of the gene in 100 US keratoconus patients and identified only one missense variant, p.(D144E), in a single case. This variant had previously been identified in unaffected controls and was considered a rare polymorphism. Similarly, Liskova et al. (2007) screened *VSX1* in 85 unrelated probands from UK families with multiple cases of keratoconus and 50 unrelated controls of mixed ethnicities. This study was the first that fully screened *VSX1* in the control cohort. The only potentially disease-causing variant identified in the case cohort was the p.(D144E) variant; however, it did not segregate with keratoconus in the pedigree. The authors conducted a meta-analysis of the p.(D144E) variant, including the results of the three previous studies (Aldave et al. 2006, Bisceglia et al. 2005, Heon et al. 2002), and demonstrated that the variant was not associated with keratoconus ($p = 0.14$). The controversial p.(P247R) variant was also identified in a control, which added further evidence that the variant does not directly cause keratoconus. Based on these findings, Liskova et al. (2007) concluded that coding variants in *VSX1* were not involved in keratoconus.

Subsequent research also excluded *VSX1* as candidate for PPCD (Gwilliam et al. 2005); however, a paper by Barbaro et al. (2006) reinvigorated the field's interest in *VSX1* in keratoconus susceptibility. Their paper analyzed adult human corneas from deceased donors and showed that *VSX1* was expressed in those with corneal defects and abrasions—dubbed wounded corneas—that were likely caused by incomplete closure of the eyelids after death. Conversely, *VSX1* expression was not detected in freshly obtained intact corneas (Barbaro et al. 2006). Using an in vitro model, the authors demonstrated that *VSX1* mRNA expression was 8–10 times higher in activated keratocytes compared to quiescent keratocytes in culture, suggesting that *VSX1* was likely to play a role in keratocyte transformation and wound healing.

Dozens of papers on *VSX1* in keratoconus have since been published, with many concluding that *VSX1* is likely to be involved in keratoconus, as well as a similar number of studies reporting no evidence of association. *VSX1* has been screened in a broad range of populations and cohorts; however, differentiating between population-specific polymorphisms and potentially disease-associated variants has been challenging. A recent meta-analysis assessed five recurrent variants identified across multiple cohorts and found no evidence for association with keratoconus (Rong et al. 2017). Furthermore, while subtle changes have been noted in the retinas of humans carrying rare missense *VSX1* variants, there is a lack of evidence for any functional

impact on corneal biology, including in *Vsx1*-null mice models (Chow et al. 2004, Litke et al. 2018). Taken together, the burden of evidence suggests that *VSX1* does not contribute to keratoconus susceptibility.

SOD1. The *superoxide dismutase 1* (*SOD1*) gene was first proposed as a candidate gene for keratoconus based on the observation that individuals with Down syndrome (trisomy 21) have a much higher prevalence of keratoconus, compared to the general population, as well as the gene's location on chromosome 21 (Udar et al. 2006). *SOD1* encodes an enzyme that is responsible for detoxifying superoxide radicals, a form of reactive oxygen species, in the cytoplasm of cells throughout the body, including the cornea. Oxidative stress from free radicals has been proposed as a mechanism involved in keratoconus pathogenesis. Udar et al. (2006) screened the exons and intron–exon junctions of *SOD1* by direct sequencing in 15 probands with keratoconus from the United States. They identified an intronic seven-base-pair (bp) deletion (reported as IVS2+50del7) in two cases that was absent in 156 older, examined controls. Segregation was confirmed in one small family but could not be determined in the other family due to lack of DNA availability. While most other studies have not observed the variant in their cohorts, an Italian study observed the deletion in two sporadic cases from a cohort of 302 patients but not in 200 controls (De Bonis et al. 2011). A Greek study identified the variant in 9 of their 33 cases, as well as in 4 of their 78 healthy controls, also demonstrating that the variant was enriched in keratoconus ($p = 0.002$; odds ratio = 6.94; 95% confidence interval = 1.96–24.58) (Moschos et al. 2013). RNA collected from one of the probands in the original study demonstrated that the variant resulted in two splice variants: one lacking exon 2 and one that lacked both exon 2 and exon 3. Both transcripts result in a shifted reading frame and inactive protein (Udar et al. 2006). The 7-bp deletion corresponds to hg19:chr21:33036248ATAAACAG>A (rs541000032), which is rare in all populations represented in gnomAD (v2.1.1) except Ashkenazi Jews, who have a minor allele frequency of around 1.5%. Much larger case-control cohorts are required to determine the true frequency of the variant in keratoconus patients. To date, gene-screen studies have not identified any potentially disease-causing variants in the coding regions of *SOD1* in a total of 597 unrelated probands or sporadic keratoconus patients (Al-Muammar et al. 2015, Gajecka et al. 2009, Lucas et al. 2018, Saee-Rad et al. 2011, Stabuc-Silih et al. 2010). This is unsurprising, as rare missense variants in *SOD1* are known to cause the neurodegenerative disease amyotrophic lateral sclerosis, which has never been associated with keratoconus.

Loci with Genome-Wide Significant Association with Keratoconus

The term genome-wide significance refers to a statistical threshold for declaring a true association, driven by the number of independent tests (or genetic variants). It is generally accepted that a p-value threshold of 5×10^{-8} provides a sufficiently robust cutoff for declaring statistically significant association in studies that assess the whole genome. Eight loci, identified through a variety of approaches, have so far been associated with keratoconus, surpassing this genome-wide significance threshold (**Figure 1**, **Table 2**).

Genome-wide association studies for keratoconus. Three genome-wide association studies (GWASs) for keratoconus have been conducted in cohorts of individuals of European descent. The first identified a suggestive association at rs3735520 ($p = 9.9 \times 10^{-7}$) located in the promotor of *HGF* (Burdon et al. 2011). Independent replication studies showed either a weak association with keratoconus or no association (Dudakova et al. 2015, Hao et al. 2015, Wang et al. 2018), and when considered together, the evidence suggests that rs3735520 is not strongly associated with

Table 2 SNPs reaching genome-wide significance for association with KC

Locus	Cytoband	SNP	A1/A2	P-value	OR (95% CI)	Original phenotype	Study
RAB3GAP1	2q21.3	rs4954218	G/T	9.3×10^{-9}	0.62[a]	KC	Bae et al. (2013)
COL4A4	2q36.3	rs2229813	G/A	1.3×10^{-12}	2.38 (1.87–3.03)	KC[c]	Rong et al. (2017)
FNDC3B	3q26.31	rs4894535	T/C	4.9×10^{-9}	1.47 (1.29–1.68)	CCT	Lu et al. (2013)
MPDZ-NFIB	9p23	rs1324183	A/C	5.0×10^{-8}	1.68 (1.22–2.30)	CCT	Sahebjada et al. (2013)
RXRA-COL5A1	9q34.3	rs1536482	G/A	2.5×10^{-8}	0.77 (0.70–0.84)	CCT	Rong et al. (2017)
PNPLA2	11p15.5	rs61876744	T/C	2.45×10^{-8}	0.59 (0.49–0.71)[b]	KC	McComish et al. (2019)
FOXO1	13q14.11	rs2721051	T/C	2.7×10^{-10}	1.62 (1.40–1.88)	CCT	Lu et al. (2013)
LOX	5q23.2	rs2956540	C/G	1.4×10^{-8}	0.71 (0.63–0.80)	KC[c]	Zhang et al. (2015)

[a]From the original study by Li et al. (2012); no 95% confidence interval reported.
[b]OR and 95% CI as reported in the discovery cohort.
[c]Identified by a candidate gene approach.
Abbreviations: A1, keratoconus-associated allele; A2, alternate allele; CCT, central corneal thickness; CI, confidence interval; KC, keratoconus; OR, odds ratio; SNP, single-nucleotide polymorphism.

keratoconus ($p = 0.027$) (Rong et al. 2017). The second GWAS for keratoconus showed a suggestive association at rs4954218 upstream of *RAB3GAP1* ($p = 1.6 \times 10^{-7}$) (Li et al. 2012) and reached the genome-wide threshold following replication and meta-analysis ($p = 5.0 \times 10^{-8}$) (Bae et al. 2013). However, the involvement of this locus in keratoconus susceptibility remains unclear, with a weaker association ($p = 8.2 \times 10^{-4}$) reported in a separate meta-analysis (Rong et al. 2017). Most recently, our group conducted a larger GWAS including 522 cases that identified a genome-wide significant association for keratoconus at rs61876744 ($p = 7.46 \times 10^{-9}$), located on chromosome 11 in the second intron of the *PNPLA2* gene (McComish et al. 2019). This association was replicated in a United States–based cohort but was not replicated in two other cohorts, although it remained significantly associated with keratoconus at the genome-wide threshold following a meta-analysis of all four cohorts ($p = 2.45 \times 10^{-8}$).

The major limitations of GWASs for keratoconus to date are the relatively small size of the cohorts and the lack of ethnic diversity. Cohorts of many thousands of cases are typically required to identify the genetic variants of small effect sizes that contribute to complex disease. These early GWASs have served to establish international collaborations that will lead to far larger studies in the near future that are better powered to detect genetic effects. All of the GWASs reported to date are in predominantly white populations of European descent (the United States and Australia). Larger studies in these populations will identify additional loci, but to fully understand the genetics of the disease, studies in other ethnic groups with different genetic diversity and higher rates of disease are clearly warranted.

Genome-wide association studies for central corneal thickness. Leveraging endophenotypes for keratoconus has been a successful strategy for identifying keratoconus-associated loci. An

endophenotype refers to a quantitative trait that is associated with the disease of interest in the population, is heritable, is measurable in both healthy and affected individuals, and is genetically correlated to the disease. Endophenotypes are hypothesized to be less complex and closer to the underlying genetics than the disease of interest, as they reflect just one of many pathophysiological pathways that contribute to disease susceptibility (Gottesman & Gould 2003). This allows the dissection of complex diseases, which directs investigators toward the relevant biological pathways and ultimately aiding the identification of functional variants and target genes. A recent study identified genetic loci for the corneal biomechanical measures corneal hysteresis and corneal resistance factor and assessed them for association with keratoconus (Khawaja et al. 2019). While the hypothesis was sound, and suggestive association was reported, larger cohorts of keratoconus patients need to be assessed to confirm the genetic links between these measures and keratoconus.

CCT is considered a strong endophenotype for keratoconus. CCT is a quantitative trait with a normal distribution in the general population (mean = 536 μm; standard deviation = 31 μm) (Doughty & Zaman 2000). Although the genetic correlation between CCT and keratoconus has not been calculated, keratoconus is associated with extremely low CCT, with a mean of 434 μm reported in keratoconic eyes (Doughty & Zaman 2000), well outside the normal variance observed in the general population. CCT is one of the most heritable human traits, with heritability estimates of up to 95% (Alsbirk 1978, Charlesworth et al. 2010, Landers et al. 2009, Toh et al. 2005, Zheng et al. 2008). Unaffected family members of individuals with keratoconus have been shown to have thinner corneas when compared to population controls (Colin et al. 1996, Steele et al. 2008), with some evidence of an autosomal-dominant pattern of inheritance (Steele et al. 2008).

Using this endophenotype approach, Lu et al. (2013) identified two CCT-associated SNPs that were also associated with keratoconus at the genome-wide significance threshold: rs2721051, downstream of *FOXO1* ($p = 2.7 \times 10^{-10}$), and rs4894535, located in an intron of *FNDC3B* ($p = 4.9 \times 10^{-9}$). Replication studies for rs2721051 (*FOXO1*) revealed that the risk (A) allele is extremely rare in the Chinese population (Hao et al. 2015, Wang et al. 2018). The association at rs2721051 was replicated in a Czech cohort (Liskova et al. 2017) but not in an Australian cohort of European descent ($p = 0.06$) (Sahebjada et al. 2013) or a Saudi Arabian cohort ($p = 0.2$) (Abu-Amero et al. 2015a), although both of these latter studies observed similar trends in allele frequency differences between cases and controls to those reported by Lu et al. (2013). The association at rs4894535 (*FNDC3B*) was not replicated in the Czech, Saudi Arabian, or Chinese studies but did show trends toward a higher frequency of the minor allele in cases compared to controls (Abu-Amero et al. 2015a, Hao et al. 2015, Liskova et al. 2017, Wang et al. 2018). Meta-analysis of studies published prior to 2017 demonstrated that both rs2721051 and rs4894535 were significantly associated with keratoconus at a genome-wide level ($p = 5.6 \times 10^{-11}$ and $p = 1.4 \times 10^{-8}$, respectively), indicating that these loci are involved in keratoconus risk (Rong et al. 2017), although there may be differences between ethnic groups. The mechanisms that drive the increased keratoconus risk at these loci remain to be elucidated.

A suggestive association for keratoconus at rs1324183 (*MPDZ-NFIB*) was first reported by Lu et al. (2013) and reached genome-wide significance following replication in an Australian cohort of European descent and meta-analysis with the original report (Sahebjada et al. 2013). The association at this SNP has since been replicated in a Han Chinese cohort from northern China (Hao et al. 2015), a Chinese cohort from Hong Kong (Wang et al. 2018), and a Czech cohort (Liskova et al. 2017), with notably larger effect sizes in the Chinese studies (3.11 and 2.22, respectively, versus 1.58). The Hong Kong study also demonstrated that rs1324183 was associated with an increased risk of severe keratoconus based on the Amsler-Krumeich classification (OR = 5.10). In

contrast, there was no evidence of association in a Saudi Arabian population (Abu-Amero et al. 2015a). A large meta-analysis conducted prior to the Czech and Hong Kong studies did not show a genome-wide significant association with keratoconus at rs1324183 ($p = 5.5 \times 10^{-3}$) (Rong et al. 2017); however, overall, the evidence suggests that this is a true association.

In addition, Lu et al. (2013) revealed suggestive associations for keratoconus at two independent SNPs near the collagen gene *COL5A1*: rs7044529 in the first intron ($p = 8.0 \times 10^{-6}$) and rs1536482, located upstream between *RXRA* and *COL5A1* ($p = 2.6 \times 10^{-7}$). Using the United States–based replication cohort from the original publication, Li et al. (2013) fine-mapped the *COL5A1* region and used an independent case-control cohort and a familial cohort for replication. Meta-analysis of the three cohorts demonstrated that rs1536482 was the only SNP that remained significantly associated with keratoconus following correction for multiple testing (Li et al. 2013). The associations with keratoconus at these loci have not been replicated in more recent studies (Abu-Amero et al. 2015a, Hao et al. 2015, Sahebjada et al. 2013, Wang et al. 2018), although a trend toward a higher frequency of the minor allele (A) at rs1536482 was observed in cases compared to controls in a Czech cohort (Liskova et al. 2017). A meta-analysis that included only the cohorts of European descent published prior to 2017 showed a genome-wide significant association for keratoconus at rs1536482 ($p = 2.5 \times 10^{-9}$) (Rong et al. 2017). In contrast, meta-analysis at rs7044529 including all cohorts except that of the more recently published Hong Kong study demonstrated an overall p-value of 7.0×10^{-3}. This locus still did not reach genome-wide significance when the analysis only included the individuals of European descent ($p = 9.9 \times 10^{-4}$) (Rong et al. 2017).

The same GWAS for CCT (Lu et al. 2013) identified a suggestive association with keratoconus at the CCT-associated SNP rs9938149, located between the genes *BANP* and *ZNF469*. However, the allele that increased keratoconus risk was the allele associated with a thicker CCT. Replication studies have only added to the confusion, with one suggesting that the association is likely to be real despite the nonintuitive direction of effect (Sahebjada et al. 2013), while others showed no evidence of association (Abu-Amero et al. 2015a, Hao et al. 2015, Liskova et al. 2017, Wang et al. 2018). When these results are combined together in a meta-analysis, excluding the study published after 2017, rs9938149 shows a suggestive association with keratoconus ($p = 1.3 \times 10^{-5}$) (Rong et al. 2017). The closest gene to rs9938149—*ZNF469*, a transcription factor gene located 162 kb downstream—was considered an appealing candidate for keratoconus based on the gene's involvement in brittle cornea syndrome type 1 (BCS1; OMIM ID 229200).

BCS1 is a rare, autosomal-recessive connective tissue disorder caused by biallelic loss-of-function variants in *ZNF469*. A key feature of this syndrome is extremely thin corneas that are prone to spontaneous rupture, indicating that *ZNF469* is important for the structural integrity of the cornea. It was therefore hypothesized that rare heterozygous coding variants in *ZNF469* could be involved in keratoconus pathogenesis. Early reports supported an enrichment of rare, heterozygous potentially pathogenic variants in *ZNF469* in European and New Zealander populations of keratoconus patients compared to ethnically matched controls (Lechner et al. 2014, Vincent et al. 2014). However, two subsequent case-control studies in cohorts of European descent showed no association with disease (Karolak et al. 2016, Lucas et al. 2017). Furthermore, the analysis of rare *ZNF469* variants in 11 families with multiple cases of keratoconus did not support the involvement of this gene in keratoconus (Davidson et al. 2015). Perhaps the strongest evidence against a causative role for heterozygous variants in *ZNF469* in keratoconus comes from the lack of clinical keratoconus in parents of children with BSC1. The parents carry rare heterozygous loss-of-function variants that are pathogenic when inherited in the homozygous or a compound heterozygous state by their affected children (Davidson et al. 2015). The more recent studies also demonstrated the highly variable nature of the *ZNF469* gene and that the frequency

of many variants in publicly available databases has been underestimated due to poor capture in early exome sequencing studies (Davidson et al. 2015, Karolak et al. 2016, Lucas et al. 2017). On the whole, the evidence suggests that rare coding variants in *ZNF469* do not cause keratoconus. If *ZNF469* is involved in keratoconus susceptibility, then it is via an alternative mechanism such as gene expression. It is also worth noting that the closest gene to a GWAS hit is not necessarily the causative gene, and therefore, the unlikely effect direction observed at the rs9938149 locus may indicate that a gene other than *ZNF469* is involved in keratoconus risk.

Genome-wide significant loci for keratoconus identified using a candidate-gene approach. Two additional SNPs, initially identified through a family-based or candidate-gene approach, have reached genome-wide significance with keratoconus: rs2956540, located in an intron of *LOX* (described above), and rs2229813, a missense variant in the collagen gene *COL4A4*. *COL4A4* encodes a constituent of type IV collagen, is a major structural component of basement membranes, and is known to be expressed in human cornea. Stabuc-Silih et al. (2009) conducted an association study in a Slovenian cohort and demonstrated that the A allele at rs2229813 [p.(V1327M)] was associated with reduced risk of keratoconus ($p < 0.0001$). This finding was replicated in a second Slovenian cohort, as well as a Greek cohort (Kokolakis et al. 2014, Stabuc-Silih et al. 2010). Conversely, an Iranian study showed that the A allele at rs2229813 was associated with increased keratoconus risk, and no evidence of association was reported in a Han Chinese study (Wang et al. 2013). When considered together, these results indicate that there was no significant association with keratoconus; however, two separate meta-analyses revealed that the A allele was protective at a genome-wide significance threshold in the European cohorts, while a nominal association with an opposite effect direction was observed in the Iranian and Han Chinese cohorts (Rong et al. 2017). This may reflect real differences in the susceptibility to keratoconus between European and non-European groups; however, this locus requires further dissection.

SUMMARY AND CONCLUSION

Keratoconus is a complex disease. We do not understand all the factors that contribute to keratoconus risk and pathogenesis, but there is clear evidence that genetics plays a role in susceptibility. In most individuals, there is likely a combination of genetic and environmental factors. In patients from extended families with early onset, severe disease, and dominant or recessive inheritance, genetics may confer almost all of the risk. Conversely, in individuals with mild disease, later onset, and no family history, environmental factors may play a much greater role. The interplay between genes and environment is yet to be understood in the context of keratoconus.

Identifying genetic risk factors for keratoconus has proved challenging. As with most complex diseases, candidate gene and linkage studies of multiple small families have failed to make major contributions to our understanding. In addition, many studies were conducted on very small cohorts and without replication or subsequent follow-up. For most reported loci, it is not clear that the association is a true effect, and for those loci that have been confirmed, mechanistic studies to understand the biology have yet to emerge. Recent advances in genomic methodologies and recruitment of ever larger patient cohorts have left the field poised for major breakthroughs. For example, detailed genomic studies of extended families with clear inheritance patterns are beginning to pinpoint specific genes, and well-powered GWASs for keratoconus and CCT have highlighted multiple risk loci. The challenge now lies in identifying the full spectrum of genetic risk and determining how specific variants contribute to disease development. Achieving these goals will improve our ability to use genetics in the diagnosis and management of keratoconus and to develop precision medicine treatments focused on the molecular cause of the disease.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors would like to thank Patricia Graham and Dr. Jac Charlesworth for constructive discussion and valuable editorial and proofing assistance.

LITERATURE CITED

Abu-Amero KK, Helwa I, Al-Muammar A, Strickland S, Hauser MA, et al. 2015a. Case-control association between CCT-associated variants and keratoconus in a Saudi Arabian population. *J. Negat. Results Biomed.* 14:10

Abu-Amero KK, Helwa I, Al-Muammar A, Strickland S, Hauser MA, et al. 2015b. Screening of the seed region of MIR184 in keratoconus patients from Saudi Arabia. *Biomed. Res. Int.* 2015:604508

Akpek EK, Jun AS, Goodman DF, Green WR, Gottsch JD. 2002. Clinical and ultrastructural features of a novel hereditary anterior segment dysgenesis. *Ophthalmology* 109:513–19

Al-Muammar AM, Kalantan H, Azad TA, Sultan T, Abu-Amero KK. 2015. Analysis of the SOD1 gene in keratoconus patients from Saudi Arabia. *Ophthalmic Genet.* 36:373–75

Aldave AJ, Yellore VS, Principe AH, Abedi G, Merrill K, et al. 2005. Candidate gene screening for posterior polymorphous dystrophy. *Cornea* 24:151–55

Aldave AJ, Yellore VS, Salem AK, Yoo GL, Rayner SA, et al. 2006. No VSX1 gene mutations associated with keratoconus. *Investig. Ophthalmol. Vis. Sci.* 47:2820–22

Alio JL, Vega-Estrada A, Sanz P, Osman AA, Kamal AM, et al. 2018. Corneal morphologic characteristics in patients with Down syndrome. *JAMA Ophthalmol.* 136:971–78

Alsbirk PH. 1978. Corneal thickness. II. Environmental and genetic factors. *Acta Ophthalmol.* 56:105–13

Bae HA, Mills RA, Lindsay RG, Phillips T, Coster DJ, et al. 2013. Replication and meta-analysis of candidate loci identified variation at RAB3GAP1 associated with keratoconus. *Investig. Ophthalmol. Vis. Sci.* 54:5132–35

Bak-Nielsen S, Ramlau-Hansen CH, Ivarsen A, Plana-Ripoll O, Hjortdal J. 2018. A nationwide population-based study of social demographic factors, associated diseases and mortality of keratoconus patients in Denmark from 1977 to 2015. *Acta Ophthalmol.* 97:497–504

Barbaro V, Di Iorio E, Ferrari S, Bisceglia L, Ruzza A, et al. 2006. Expression of VSX1 in human corneal keratocytes during differentiation into myofibroblasts in response to wound healing. *Investig. Ophthalmol. Vis. Sci.* 47:5243–50

Bawazeer AM, Hodge WG, Lorimer B. 2000. Atopy and keratoconus: a multivariate analysis. *Br. J. Ophthalmol.* 84:834–36

Bisceglia L, Ciaschetti M, De Bonis P, Campo PA, Pizzicoli C, et al. 2005. VSX1 mutational analysis in a series of Italian patients affected by keratoconus: detection of a novel mutation. *Investig. Ophthalmol. Vis. Sci.* 46:39–45

Bisceglia L, De Bonis P, Pizzicoli C, Fischetti L, Laborante A, et al. 2009. Linkage analysis in keratoconus: replication of locus 5q21.2 and identification of other suggestive loci. *Investig. Ophthalmol. Vis. Sci.* 50:1081–86

Brancati F, Valente EM, Sarkozy A, Feher J, Castori M, et al. 2004. A locus for autosomal dominant keratoconus maps to human chromosome 3p14-q13. *J. Med. Genet.* 41:188–92

Burdon KP, Coster DJ, Charlesworth JC, Mills RA, Laurie KJ, et al. 2008. Apparent autosomal dominant keratoconus in a large Australian pedigree accounted for by digenic inheritance of two novel loci. *Hum. Genet.* 124:379–86

Burdon KP, Macgregor S, Bykhovskaya Y, Javadiyan S, Li X, et al. 2011. Association of polymorphisms in the hepatocyte growth factor gene promoter with keratoconus. *Investig. Ophthalmol. Vis. Sci.* 52:8514–19

Bykhovskaya Y, Caiado Canedo AL, Wright KW, Rabinowitz YS. 2015a. C.57 C>T mutation in MIR 184 is responsible for congenital cataracts and corneal abnormalities in a five-generation family from Galicia, Spain. *Ophthalmic Genet*. 36:244–47

Bykhovskaya Y, Li X, Epifantseva I, Haritunians T, Siscovick D, et al. 2012. Variation in the lysyl oxidase (LOX) gene is associated with keratoconus in family-based and case-control studies. *Investig. Ophthalmol. Vis. Sci.* 53:4152–57

Bykhovskaya Y, Li X, Taylor KD, Haritunians T, Rotter JI, Rabinowitz YS. 2014. Linkage analysis of high-density SNPs confirms keratoconus locus at 5q chromosomal region. *Ophthalmic Genet*. 37:109–10

Bykhovskaya Y, Seldin MF, Liu Y, Ransom M, Li X, Rabinowitz YS. 2015b. Independent origin of c.57C>T mutation in MIR184 associated with inherited corneal and lens abnormalities. *Ophthalmic Genet*. 36:95–97

Cagil N, Ugurlu N, Sahan CY, Sevli S. 2017. Lack of MIR143, MIR145, MIR184, MIR1224, and MIR29b1 mutations in keratoconus pathogenesis. *Turk. J. Med. Sci.* 47:1669–71

Chao CC, Butala SM, Herp A. 1988. Studies on the isolation and composition of human ocular mucin. *Exp. Eye Res.* 47:185–96

Charlesworth J, Kramer PL, Dyer T, Diego V, Samples JR, et al. 2010. The path to open-angle glaucoma gene discovery: endophenotypic status of intraocular pressure, cup-to-disc ratio, and central corneal thickness. *Investig. Ophthalmol. Vis. Sci.* 51:3509–14

Chow RL, Volgyi B, Szilard RK, Ng D, McKerlie C, et al. 2004. Control of late off-center cone bipolar cell differentiation and visual signaling by the homeobox gene Vsx1. *PNAS* 101:1754–59

Colin J, Sale Y, Malet F, Cochener B. 1996. Inferior steepening is associated with thinning of the inferotemporal cornea. *J. Refract. Surg.* 12:697–99

Cote JF, Motoyama AB, Bush JA, Vuori K. 2005. A novel and evolutionarily conserved PtdIns(3,4,5)P3-binding domain is necessary for DOCK180 signalling. *Nat. Cell Biol.* 7:797–807

Cote JF, Vuori K. 2006. In vitro guanine nucleotide exchange activity of DHR-2/DOCKER/CZH2 domains. *Methods Enzymol.* 406:41–57

Cozma I, Atherley C, James NJ. 2005. Influence of ethnic origin on the incidence of keratoconus and associated atopic disease in Asian and white patients. *Eye* 19:924–25; author reply 925–26

Czugala M, Karolak JA, Nowak DM, Polakowski P, Pitarque J, et al. 2012. Novel mutation and three other sequence variants segregating with phenotype at keratoconus 13q32 susceptibility locus. *Eur. J. Hum. Genet.* 20:389–97

Dash DP, Silvestri G, Hughes AE. 2006. Fine mapping of the keratoconus with cataract locus on chromosome 15q and candidate gene analysis. *Mol. Vis.* 12:499–505

Davidson AE, Borasio E, Liskova P, Khan AO, Hassan H, et al. 2015. Brittle cornea syndrome ZNF469 mutation carrier phenotype and segregation analysis of rare ZNF469 variants in familial keratoconus. *Investig. Ophthalmol. Vis. Sci.* 56:578–86

De Bonis P, Laborante A, Pizzicoli C, Stallone R, Barbano R, et al. 2011. Mutational screening of VSX1, SPARC, SOD1, LOX, and TIMP3 in keratoconus. *Mol. Vis.* 17:2482–94

Dharmaraj S, Leroy BP, Sohocki MM, Koenekoop RK, Perrault I, et al. 2004. The phenotype of Leber congenital amaurosis in patients with AIPL1 mutations. *Arch. Ophthalmol.* 122:1029–37

Dimasi DP, Hewitt AW, Kagame K, Ruvama S, Tindyebwa L, et al. 2011. Ethnic and mouse strain differences in central corneal thickness and association with pigmentation phenotype. *PLOS ONE* 6:e22103

Doughty MJ, Zaman ML. 2000. Human corneal thickness and its impact on intraocular pressure measures: a review and meta-analysis approach. *Surv. Ophthalmol.* 44:367–408

Dudakova L, Palos M, Jirsova K, Stranecky V, Krepelova A, et al. 2015. Validation of rs2956540:G>C and rs3735520:G>A association with keratoconus in a population of European descent. *Eur. J. Hum. Genet.* 23:1581–83

Durst R, Sauls K, Peal DS, deVlaming A, Toomer K, et al. 2015. Mutations in DCHS1 cause mitral valve prolapse. *Nature* 525:109–13

Farzadfard A, Nassiri N, Moghadam TN, Paylakhi SH, Elahi E. 2016. Screening for MIR184 mutations in Iranian patients with keratoconus. *J. Ophthalmic Vis. Res.* 11:3–7

Froukh T, Hawwari A. 2019. Autosomal recessive non-syndromic keratoconus: homozygous frameshift variant in the candidate novel gene GALNT14. *Curr. Mol. Med.* 19:683–87

Gajecka M, Radhakrishna U, Winters D, Nath SK, Rydzanicz M, et al. 2009. Localization of a gene for keratoconus to a 5.6-Mb interval on 13q32. *Investig. Ophthalmol. Vis. Sci.* 50:1531–39

Gordon-Shaag A, Millodot M, Essa M, Garth J, Ghara M, Shneor E. 2013. Is consanguinity a risk factor for keratoconus? *Optom. Vis. Sci.* 90:448–54

Gordon-Shaag A, Millodot M, Kaiserman I, Sela T, Barnett Itzhaki G, et al. 2015a. Risk factors for keratoconus in Israel: a case-control study. *Ophthalmic Physiol. Opt.* 35:673–81

Gordon-Shaag A, Millodot M, Shneor E, Liu Y. 2015b. The genetic and environmental factors for keratoconus. *Biomed. Res. Int.* 2015:795738

Gorskova EN, Sevost'ianov EN. 1998. Epidemiology of keratoconus in the Urals. *Vestn. Oftalmol.* 114:38–40

Gottesman II, Gould TD. 2003. The endophenotype concept in psychiatry: etymology and strategic intentions. *Am. J. Psychiatry* 160:636–45

Gwilliam R, Liskova P, Filipec M, Kmoch S, Jirsova K, et al. 2005. Posterior polymorphous corneal dystrophy in Czech families maps to chromosome 20 and excludes the VSX1 gene. *Investig. Ophthalmol. Vis. Sci.* 46:4480–84

Hameed A, Khaliq S, Ismail M, Anwar K, Ebenezer ND, et al. 2000. A novel locus for Leber congenital amaurosis (LCA4) with anterior keratoconus mapping to chromosome 17p13. *Investig. Ophthalmol. Vis. Sci.* 41:629–33

Hao XD, Chen P, Chen ZL, Li SX, Wang Y. 2015. Evaluating the association between keratoconus and reported genetic loci in a Han Chinese population. *Ophthalmic Genet.* 36:132–36

Hashemi H, Heydarian S, Hooshmand E, Saatchi M, Yekta A, et al. 2019. The prevalence and risk factors for keratoconus: a systematic review and meta-analysis. *Cornea* 39:263–70

Heon E, Greenberg A, Kopp KK, Rootman D, Vincent AL, et al. 2002. VSX1: a gene for posterior polymorphous dystrophy and keratoconus. *Hum. Mol. Genet.* 11:1029–36

Hughes AE, Bradley DT, Campbell M, Lechner J, Dash DP, et al. 2011. Mutation altering the miR-184 seed region causes familial keratoconus with cataract. *Am. J. Hum. Genet.* 89:628–33

Hughes AE, Dash DP, Jackson AJ, Frazer DG, Silvestri G. 2003. Familial keratoconus with cataract: linkage to the long arm of chromosome 15 and exclusion of candidate genes. *Investig. Ophthalmol. Vis. Sci.* 44:5063–66

Ihalainen A. 1986. Clinical and epidemiological features of keratoconus genetic and external factors in the pathogenesis of the disease. *Acta Ophthalmol. Suppl.* 178:1–64

Iliff BW, Riazuddin SA, Gottsch JD. 2012. A single-base substitution in the seed region of miR-184 causes EDICT syndrome. *Investig. Ophthalmol. Vis. Sci.* 53:348–53

Jun AS, Broman KW, Do DV, Akpek EK, Stark WJ, Gottsch JD. 2002. Endothelial dystrophy, iris hypoplasia, congenital cataract, and stromal thinning (edict) syndrome maps to chromosome 15q22.1-q25.3. *Am. J. Ophthalmol.* 134:172–76

Kalkan Akcay E, Akcay M, Uysal BS, Kosekahya P, Aslan AN, et al. 2014. Impaired corneal biomechanical properties and the prevalence of keratoconus in mitral valve prolapse. *J. Ophthalmol.* 2014:402193

Karolak JA, Gambin T, Rydzanicz M, Szaflik JP, Polakowski P, et al. 2016. Evidence against ZNF469 being causative for keratoconus in Polish patients. *Acta Ophthalmol.* 94:289–94

Karolak JA, Rydzanicz M, Ginter-Matuszewska B, Pitarque JA, Molinari A, et al. 2015. Variant c.2262A>C in DOCK9 leads to exon skipping in keratoconus family. *Investig. Ophthalmol. Vis. Sci.* 56:7687–90

Khawaja AP, Rojas Lopez KE, Hardcastle AJ, Hammond CJ, Liskova P, et al. 2019. Genetic variants associated with corneal biomechanical properties and potentially conferring susceptibility to keratoconus in a genome-wide association study. *JAMA Ophthalmol.* 137:1005–12

Klyce SD. 1984. Computer-assisted corneal topography: high-resolution graphic presentation and analysis of keratoscopy. *Investig. Ophthalmol. Vis. Sci.* 25:1426–35

Klyce SD. 2009. Chasing the suspect: keratoconus. *Br. J. Ophthalmol.* 93:845–47

Kokolakis NS, Gazouli M, Chatziralli IP, Koutsandrea C, Gatzioufas Z, et al. 2014. Polymorphism analysis of COL4A3 and COL4A4 genes in Greek patients with keratoconus. *Ophthalmic Genet.* 35:226–28

Kosker M, Suri K, Hammersmith KM, Nassef AH, Nagra PK, Rapuano CJ. 2014. Another look at the association between diabetes and keratoconus. *Cornea* 33:774–79

Kriszt A, Losonczy G, Berta A, Vereb G, Takacs L. 2014. Segregation analysis suggests that keratoconus is a complex non-Mendelian disease. *Acta Ophthalmol.* 92:e562–68

Kulkarni K, Yang J, Zhang Z, Barford D. 2011. Multiple factors confer specific Cdc42 and Rac protein activation by dedicator of cytokinesis (DOCK) nucleotide exchange factors. *J. Biol. Chem.* 286:25341–51

Kuo IC, Broman A, Pirouzmanesh A, Melia M. 2006. Is there an association between diabetes and keratoconus? *Ophthalmology* 113:184–90

Landers JA, Hewitt AW, Dimasi DP, Charlesworth JC, Straga T, et al. 2009. Heritability of central corneal thickness in nuclear families. *Investig. Ophthalmol. Vis. Sci.* 50:4087–90

Le Tourneau T, Merot J, Rimbert A, Le Scouarnec S, Probst V, et al. 2018. Genetics of syndromic and non-syndromic mitral valve prolapse. *Heart* 104:978–84

Lechner J, Bae HA, Guduric-Fuchs J, Rice A, Govindarajan G, et al. 2013. Mutational analysis of MIR184 in sporadic keratoconus and myopia. *Investig. Ophthalmol. Vis. Sci.* 54:5266–72

Lechner J, Porter LF, Rice A, Vitart V, Armstrong DJ, et al. 2014. Enrichment of pathogenic alleles in the brittle cornea gene, ZNF469, in keratoconus. *Hum. Mol. Genet.* 23:5527–35

Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, et al. 2016. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 536:285–91

Li X, Bykhovskaya Y, Canedo AL, Haritunians T, Siscovick D, et al. 2013. Genetic association of COL5A1 variants in keratoconus patients suggests a complex connection between corneal thinning and keratoconus. *Investig. Ophthalmol. Vis. Sci.* 54:2696–704

Li X, Bykhovskaya Y, Haritunians T, Siscovick D, Aldave A, et al. 2012. A genome-wide association study identifies a potential novel gene locus for keratoconus, one of the commonest causes for corneal transplantation in developed countries. *Hum. Mol. Genet.* 21:421–29

Li X, Rabinowitz YS, Tang YG, Picornell Y, Taylor KD, et al. 2006. Two-stage genome-wide linkage scan in keratoconus sib pair families. *Investig. Ophthalmol. Vis. Sci.* 47:3791–95

Liskova P, Dudakova L, Krepelova A, Klema J, Hysi PG. 2017. Replication of SNP associations with keratoconus in a Czech cohort. *PLOS ONE* 12:e0172365

Liskova P, Ebenezer ND, Hysi PG, Gwilliam R, El-Ashry MF, et al. 2007. Molecular analysis of the VSX1 gene in familial keratoconus. *Mol. Vis.* 13:1887–91

Liskova P, Hysi PG, Waseem N, Ebenezer ND, Bhattacharya SS, Tuft SJ. 2010. Evidence for keratoconus susceptibility locus on chromosome 14: a genome-wide linkage screen using single-nucleotide polymorphism markers. *Arch. Ophthalmol.* 128:1191–95

Litke AM, Samuelson S, Delaney KR, Sauve Y, Chow RL. 2018. Investigating the pathogenicity of VSX1 missense mutations and their association with corneal disease. *Investig. Ophthalmol. Vis. Sci.* 59:5824–35

Lu Y, Vitart V, Burdon KP, Khor CC, Bykhovskaya Y, et al. 2013. Genome-wide association analyses identify multiple loci associated with central corneal thickness and keratoconus. *Nat. Genet.* 45:155–63

Lucas SEM, Zhou T, Blackburn NB, Mills RA, Ellis J, et al. 2017. Rare, potentially pathogenic variants in ZNF469 are not enriched in keratoconus in a large Australian cohort of European descent. *Investig. Ophthalmol. Vis. Sci.* 58:6248–56

Lucas SEM, Zhou T, Blackburn NB, Mills RA, Ellis J, et al. 2018. Rare, potentially pathogenic variants in 21 keratoconus candidate genes are not enriched in cases in a large Australian cohort of European descent. *PLOS ONE* 13:e0199178

Maeda N, Klyce SD, Smolek MK, Thompson HW. 1994. Automated keratoconus screening with corneal topography analysis. *Investig. Ophthalmol. Vis. Sci.* 35:2749–57

Maharana PK, Sharma N, Vajpayee RB. 2013. Acute corneal hydrops in keratoconus. *Indian J. Ophthalmol.* 61:461–64

Mantelli F, Argueso P. 2008. Functions of ocular surface mucins in health and disease. *Curr. Opin. Allergy Clin. Immunol.* 8:477–83

McComish B, Sahebjada S, Bykhovskaya Y, Willoughby C, Richardson A, et al. 2019. Association of genetic variation with keratoconus. *JAMA Ophthalmol.* 138:174–81

McMahon TT, Kim LS, Fishman GA, Stone EM, Zhao XC, et al. 2009. CRB1 gene mutations are associated with keratoconus in patients with Leber congenital amaurosis. *Investig. Ophthalmol. Vis. Sci.* 50:3185–87

McMonnies CW. 2009. Mechanisms of rubbing-related corneal trauma in keratoconus. *Cornea* 28:607–15

McMonnies CW, Boneham GC. 2003. Keratoconus, allergy, itch, eye-rubbing and hand-dominance. *Clin. Exp. Optom.* 86:376–84

Meller N, Irani-Tehrani M, Kiosses WB, Del Pozo MA, Schwartz MA. 2002. Zizimin1, a novel Cdc42 activator, reveals a new GEF domain for Rho proteins. *Nat. Cell Biol.* 4:639–47

Merdler I, Hassidim A, Sorkin N, Shapira S, Gronovich Y, Korach Z. 2015. Keratoconus and allergic diseases among Israeli adolescents between 2005 and 2013. *Cornea* 34:525–29

Moschos MM, Kokolakis N, Gazouli M, Chatziralli IP, Droutsas D, et al. 2013. Polymorphism analysis of VSX1 and SOD1 genes in Greek patients with keratoconus. *Ophthalmic Genet.* 36:13–17

Naderan M, Naderan M, Rezagholizadeh F, Zolfaghari M, Pahlevani R, Rajabi MT. 2014. Association between diabetes and keratoconus: a case-control study. *Cornea* 33:1271–73

Naderan M, Rajabi MT, Zarrinbakhsh P, Bakhshi A. 2017. Effect of allergic diseases on keratoconus severity. *Ocul. Immunol. Inflamm.* 25:418–23

Naderan M, Shoar S, Rezagholizadeh F, Zolfaghari M, Naderan M. 2015. Characteristics and associations of keratoconus patients. *Cont. Lens Anterior Eye* 38:199–205

Nemet AY, Vinker S, Bahar I, Kaiserman I. 2010. The association of keratoconus with immune disorders. *Cornea* 29:1261–64

Nielsen K, Birkenkamp-Demtroder K, Ehlers N, Orntoft TF. 2003. Identification of differentially expressed genes in keratoconus epithelium analyzed on microarrays. *Investig. Ophthalmol. Vis. Sci.* 44:2466–76

Nielsen K, Hjortdal J, Aagaard Nohr E, Ehlers N. 2007. Incidence and prevalence of keratoconus in Denmark. *Acta Ophthalmol. Scand.* 85:890–92

Nowak DM, Karolak JA, Kubiak J, Gut M, Pitarque JA, et al. 2013. Substitution at IL1RN and deletion at SLC4A11 segregating with phenotype in familial keratoconus. *Investig. Ophthalmol. Vis. Sci.* 54:2207–15

Owens H, Gamble G. 2003. A profile of keratoconus in New Zealand. *Cornea* 22:122–25

Rabinowitz YS. 1998. Keratoconus. *Surv. Ophthalmol.* 42:297–319

Rabinowitz YS. 2003. The genetics of keratoconus. *Ophthalmol. Clin. North Am.* 16:607–20

Rabinowitz YS, Rasheed K. 1999. KISA% index: a quantitative videokeratography algorithm embodying minimal topographic criteria for diagnosing keratoconus. *J. Cataract Refract. Surg.* 25:1327–35

Romero-Jimenez M, Santodomingo-Rubido J, Wolffsohn JS. 2010. Keratoconus: a review. *Cont. Lens Anterior Eye* 33:157–66; quiz 205

Rong SS, Ma STU, Yu XT, Ma L, Chu WK, et al. 2017. Genetic associations for keratoconus: a systematic review and meta-analysis. *Sci. Rep.* 7:4620

Ryan DG, Oliveira-Fernandes M, Lavker RM. 2006. MicroRNAs of the mammalian eye display distinct and overlapping tissue specificity. *Mol. Vis.* 12:1175–84

Saad A, Gatinel D. 2010. Topographic and tomographic properties of forme fruste keratoconus corneas. *Investig. Ophthalmol. Vis. Sci.* 51:5546–55

Saee-Rad S, Hashemi H, Miraftab M, Noori-Daloii MR, Chaleshtori MH, et al. 2011. Mutation analysis of VSX1 and SOD1 in Iranian patients with keratoconus. *Mol. Vis.* 17:3128–36

Sahebjada S, Schache M, Richardson AJ, Snibson G, MacGregor S, et al. 2013. Evaluating the association between keratoconus and the corneal thickness genes in an independent Australian population. *Investig. Ophthalmol. Vis. Sci.* 54:8224–28

Seiler T, Huhle S, Spoerl E, Kunath H. 2000. Manifest diabetes and keratoconus: a retrospective case-control study. *Graefe's Arch. Clin. Exp. Ophthalmol.* 238:822–25

Shneor E, Millodot M, Blumberg S, Ortenberg I, Behrman S, Gordon-Shaag A. 2013. Characteristics of 244 patients with keratoconus seen in an optometric contact lens practice. *Clin. Exp. Optom.* 96:219–24

Sohocki MM, Bowne SJ, Sullivan LS, Blackshaw S, Cepko CL, et al. 2000. Mutations in a new photoreceptor-pineal gene on 17p cause Leber congenital amaurosis. *Nat. Genet.* 24:79–83

Stabuc-Silih M, Ravnik-Glavac M, Glavac D, Hawlina M, Strazisar M. 2009. Polymorphisms in COL4A3 and COL4A4 genes associated with keratoconus. *Mol. Vis.* 15:2848–60

Stabuc-Silih M, Strazisar M, Ravnik-Glavac M, Hawlina M, Glavac D. 2010. Genetics and clinical characteristics of keratoconus. *Acta Dermatovenerol. Alp. Pannonica Adriat.* 19:3–10

Steele TM, Fabinyi DC, Couper TA, Loughnan MS. 2008. Prevalence of Orbscan II corneal abnormalities in relatives of patients with keratoconus. *Clin. Exp. Ophthalmol.* 36:824–30

Tang YG, Rabinowitz YS, Taylor KD, Li X, Hu M, et al. 2005. Genomewide linkage scan in a multigeneration Caucasian pedigree identifies a novel locus for keratoconus on chromosome 5q14.3-q21.1. *Genet. Med.* 7:397–405

Toh T, Liew SH, MacKinnon JR, Hewitt AW, Poulsen JL, et al. 2005. Central corneal thickness is highly heritable: the twin eye studies. *Investig. Ophthalmol. Vis. Sci.* 46:3718–22

Torres Netto EA, Al-Otaibi WM, Hafezi NL, Kling S, Al-Farhan HM, et al. 2018. Prevalence of keratoconus in paediatric patients in Riyadh, Saudi Arabia. *Br. J. Ophthalmol.* 102:1436–41

Tyynismaa H, Sistonen P, Tuupanen S, Tervo T, Dammert A, et al. 2002. A locus for autosomal dominant keratoconus: linkage to 16q22.3-q23.1 in Finnish families. *Investig. Ophthalmol. Vis. Sci.* 43:3160–64

Udar N, Atilano SR, Brown DJ, Holguin B, Small K, et al. 2006. SOD1: a candidate gene for keratoconus. *Investig. Ophthalmol. Vis. Sci.* 47:3345–51

Vazirani J, Basu S. 2013. Keratoconus: current perspectives. *Clin. Ophthalmol.* 7:2019–30

Vincent AL, Jordan CA, Cadzow MJ, Merriman TR, McGhee CN. 2014. Mutations in the zinc finger protein gene, ZNF469, contribute to the pathogenesis of keratoconus. *Investig. Ophthalmol. Vis. Sci.* 55:5629–35

Wang Y, Jin T, Zhang X, Wei W, Cui Y, et al. 2013. Common single nucleotide polymorphisms and keratoconus in the Han Chinese population. *Ophthalmic Genet.* 34:160–66

Wang Y, Rabinowitz YS, Rotter JI, Yang H. 2000. Genetic epidemiological study of keratoconus: evidence for major gene determination. *Am. J. Med. Genet.* 93:403–9

Wang YM, Ma L, Lu SY, Chan TCY, Yam JCS, et al. 2018. Analysis of multiple genetic loci reveals MPDZ-NF1B rs1324183 as a putative genetic marker for keratoconus. *Br. J. Ophthalmol.* 102:1736–41

Weed KH, MacEwen CJ, Giles T, Low J, McGhee CN. 2008. The Dundee University Scottish Keratoconus study: demographics, corneal signs, associated diseases, and eye rubbing. *Eye* 22:534–41

Woodward MA, Blachley TS, Stein JD. 2016. The association between sociodemographic factors, common systemic diseases, and keratoconus: an analysis of a nationwide health care claims database. *Ophthalmology* 123:457–65.e2

Ye C, Ng PK, Jhanji V. 2014. Optical quality assessment in normal and forme fruste keratoconus eyes with a double-pass system: a comparison and variability study. *Br. J. Ophthalmol.* 98:1478–83

Zadnik K, Barr JT, Edrington TB, Everett DF, Jameson M, et al. 1998. Baseline findings in the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) study. *Investig. Ophthalmol. Vis. Sci.* 39:2537–46

Zhang J, Zhang L, Hong J, Wu D, Xu J. 2015. Association of common variants in LOX with keratoconus: a meta-analysis. *PLOS ONE* 10:e0145815

Zheng Y, Ge J, Huang G, Zhang J, Liu B, et al. 2008. Heritability of central corneal thickness in Chinese: the Guangzhou Twin Eye study. *Investig. Ophthalmol. Vis. Sci.* 49:4303–7