Chapter

Molecular Genetics of Keratoconus: Clinical Implications

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Abstract

Occurrence of keratoconus is pan-ethnic with reported prevalence ranging widely from 1:400 to about 1:8000, higher in Asian than Western populations. Its genetics is complex with undefined pattern of inheritance. Familial traits are also known. More than 50 gene loci and 200 variants are associated with keratoconus, some through association studies with quantitative traits of cornea features including curvature and central thickness. Environmental, behavioral, and epigenetic factors are also involved in the etiology, likely interactively with genetic susceptibility. Regardless of sex and age of disease onset, clinical courses and responses to treatment vary. Keratoconus is a major cause of cornea transplantation and is potentially blinding. Currently collagen cross-linking provides effective treatment although responses from some patients can be unpredictable with complications. Early diagnosis is vital to obtain good treatment outcome, but in many patients early signs and symptoms are not obvious. While there are potential biomarkers, reliable presymptomatic detection and prediction of treatment response may require multitude of gene variants, cornea properties, and external risk factors.

Keywords: keratoconus, genetic markers, clinical implications

1. Introduction

Keratoconus is a progressive corneal disorder involving cone-shaped protrusion and thinning of the central or paracentral cornea, leading to various degrees of visual impairment including astigmatism and even to blindness [1]. Occurrence is usually bilateral but asymmetric between the two eyes, resulting in lopsided visual dysfunction and photophobia with asymmetric progressions and severities of the two eyes in some patients. Many patients started with disease in one eye. Precise age of disease onset is hard to be determined, but it is known to occur mostly in late teenage to early adulthood. Keratoconus is a major cause of visual impairment in young adults in most populations [2]. In one early study, 41% of the keratoconus patients were unilateral at the time of diagnosis [3]. Clinical symptoms and signs range from mild subclinical "forme fruste" or suspect keratoconus to severe and progressive form [4]. In advanced keratoconus, patients may have a v-shaped indentation of the lower eyelid on downgaze caused by a large protuberant cone [5]. Other complications may include apical thinning and irregular astigmatism. Corneal scar and even perforation can happen. Blindness is the eventual consequence to some patients.

The underlying histopathology include reductions in epithelial and stromal keratocytes and collagen contents [6] with degradations of corneal membranes and

extracellular matrix. Abnormal mitochondrial functions causing cell death and deranged lipid metabolism are associated with stromal degeneration and disrupted epithelial integrity [7]. The direct causes of pathology are not known. The molecular mechanism of keratoconus has not been identified. Since it is a progressive disease, early detection is important for appropriate treatment in order to avoid serious consequence. A protocol to identify "high-risk" individuals and genetic testing for pre-symptomatic diagnosis would be exceeding helpful.

2. Multi-factorial etiology

The etiology is complex and elusive, affected by interactive environmental and genetic factors [8]. There is association of developmental keratoconus with allergic diseases, eczema, asthma, and hay fever [9, 10]. Contact lens, excessive ultraviolet light, and persistent eye rubbing are risk factors [11, 12] but with no proven and direct cause-and-effect relationships. Links with systemic diseases have been reported [13], including Ehlers-Danlos syndrome and Down syndrome [14]. In an Italian family with osteogenesis imperfecta, which is a connective tissue disorder caused by defects in genes encoding type 1 collagen, ocular features of keratoconus were detected [15]. In Leber congenital amaurosis (LCA), keratoconus has been reported in patients from Pakistan [16, 17], Israel [18], and Australia [19], while LCA itself also features severe retinal dystrophy leading to vision loss. But reported studies are not consistent. In 233 Chinese keratoconus patients in Qingdao located in northern China, 20 (0.86%) of them had had Down syndrome [20]. However, no keratoconus was found in 140 Down syndrome children in Hong Kong in southern China [21] nor in 60 Malaysian [22] or 123 Korean children with Down syndrome [23].

There is currently no causative gene known for keratoconus that causes disease directly [24]. A repertoire of susceptibility genes has been identified with about 200 polymorphisms in more than 50 genes or loci that confer genetic risk to keratoconus [24]. But the available genetic information is insufficient to establish the genetic architecture of keratoconus. No specific pathways have been confirmed. There are, however, clinical implications from studying the keratoconus associated genes. Evidences are in collections for establishment of polygenic risk marker.

3. Timely diagnosis for treatment

Onset of disease is difficult to be determined. Very often patients are not aware of visual symptoms until later stage where ocular discomfort and vision dysfunction becomes obvious. On presentation, slit lamp is capable to detect Fleisher's ring, Vogt's striae, central or paracentral stromal thinning, corneal hydrops and central scarring. But under the slit lamp, early signs are always not obvious [5]. More sophisticated investigative technologies of keratometry, corneal topography and optical coherence tomography provide more sensitive and reliable detection of early keratoconus features on the corneal surface, thickness and curvature. Corneal biomechanical properties can now be evaluated by non-tomographical Scheimpflug imaging and non-tomographical technologies, which are capable to differentiate normal, forme fruste, and keratoconus eyes [25]. In some patients subclinical conditions can be detected [26].

Currently there is no complete cure for keratoconus. At the early stage, vision is usually correctable by spectacles or contact lens. Semi-circular plastic inserts as intrastromal corneal ring segments help reduce astigmatism [27, 28]. However, cornea transplantation is required for severe and progressive keratoconus, which is

a major cause for cornea transplantations in many countries [29]. Different types of keratoplasty have been conducted for kerotoconus, penetrating keratoplasty, epikeratoplasty, and deep anterior lamellar keratoplasty (DALK). The latter has the advantage of endothelium preservation [30]. In recent years collagen crosslinking (CXL) has been proved to be safe and effective, in inhibiting, halting, or even reverting to some extent keratoconus progression in a high proportion of patients once correctly diagnosed [31]. There has been decrease in keratoplasty after the advent of collagen crosslinking CXL combines ultraviolet irradiation light and a photosensitizer, such as riboflavin, to strengthen the inter- and intra- crosslinks in the cornea. In 2016 the US Food and Drug Administration (FDA) approved the use of riboflavin and UV for progressive keratoconus by corneal collagen cross-linking [http://avedro.com/press-releases/avedro-receives-fda-approval/]. Over the years, the CXL procedure has been vigorously studied and improved [32, 33]. Meanwhile, for the most effective treatment with best visual outcome, accurate and early detection is mandatory [34]. Younger patients, especially those with a steep maximum keratometry, are at higher risk of disease progression than older patients. The younger the age of the patient, the better is the treatment effect, according to a recent systematic review and meta-analysis involving 11,529 eyes [35]. Early or asymptomatic diagnosis is therefore vital. Recent advances in ophthalmic tomographic imaging and determination of dynamic properties have enabled reliable diagnosis based on early signs [25, 35]. Often, most patients are presented late for ophthalmic consultation and investigations. Prior patients' awareness or notifications of signs and symptoms, genetic testing, if available, would provide diagnosis before symptoms surface [36].

4. Epidemiology and ethnic variations

Occurrence of keratoconus is pan-ethnic and global with a wide range of prevalence traditionally reported from 50 to 230 per 100,000 [1, 3, 37, 38]. A recent meta-analysis of 29 studies up to June 2018 from 15 countries involving over 50 million subjects reported a global prevalence of 138 per 100,000 [39]. There are obvious ethnic variations. For whites the prevalence had been estimated to be 50 in 100,000 [1], whereas blacks and Latinos have approximately 50 percent higher risk of having keratoconus than whites [13]. Asians have higher incidence and prevalence, as well as earlier onset and faster progression than other ethnicities [13, 40].

4.1 Ethnic diversities

In the USA, the overall prevalence was estimated to be 54 per 100,000 according to an early report in 1986 [3]. In a 5-year dataset between 1999 and 2003 for Medicare beneficiaries claiming for keratoconus, the average prevalence was 17.5/100,000 [41]. The records included whites, blacks and Hispanics in ethnicities. There were more whites than other races among the claims. In Denmark, the National Patient Registry recorded 86 keratoconus patients per 100,000 during an 11-year period from 1995 to 2005 [42]. In a study in the United Kingdom, the respective prevalence for Asians (mostly Indians) and Caucasians was 229 and 57 per 100,000, respectively, and corresponding age of diagnosis was 22.3 and 26.5 years [43]. Consistent results were reported in two latter studies comparing Pakistanis and Caucasians in the United Kingdom indicating greater prevalence in Asians by 4.4–7.5 times than Caucasians [44, 45]. In the Middle East in an Iranian population, a prevalence of about 25 per 100,000 and age of diagnosis at 27.1 ± 9.3 years were reported [46]. But in a recent study on a rural population in Iran, a very high prevalence of 4000 per 100,000 was found [47]. In a hospital

based study in Saudi Arabia, the incidence was 20 per 100,000 in young patients age ranged from 8 to 24 years, with more than half (54%) of patients classified as advanced keratoconus [48]. In India, onset of disease has been reported to happen at a younger age and progresses more rapidly [49]. In a study totaling 5200 Indian patients, the average age of presentation was 21.5 years with 1970 patients (37.9%) having an onset of disease before 20 years of age. The overall prevalence was very high at 5200/100,000 (5.2%) [50]. In a rural population in central India, a slightly lower prevalence of 2300/100,000 was recorded [51]. In huge contrast, the prevalence in Japanese was low: 12 in 100,000 males and 5.6 in 100,000 females [52]. In Chinese, a population based study in Beijing for an elder population of 3468 individuals aged 50–93 years, steep cornea/keratoconus occurred in 33 persons, giving a prevalence of 950 in 100,000 [53]. In this study steep cornea/keratoconus was defined as corneal refractive power equal to or greater than 48 diopters according to optical low-coherence reflectometry. In a study 2 years later in Singapore in people older than 40 years, the prevalence of steep cornea was comparable in Malays (606 in 100,000), Indians (1000 in 100,000), and Chinese (500 in 100,000) (0.5%) (95% CI 0.3–0.8%) [54].

4.2 Basis for ethnic diversities

A summary of reported studies (**Table 1**) shows in general higher prevalence in Asians than Caucasians, with disease started earlier and severe. But occurrence at Japanese is low. There are also vast differences in the same ethnic group. Environmental factors and investigative criteria other than genetics would affect the reported occurrence of keratoconus. The very wide range of keratoconus prevalence and incidence may be a result of non-uniform diagnostic criteria applied in different studies. Another cause may be genetic variations among different ethnic populations. There is a significant role of ethnicity. Hence rigorous, multiethnic, well-organized, and population-based epidemiological studies with large sample sizes for keratoconus are needed. Nevertheless, in addition to ethnicity, currently reported epidemiologic studies indicate that potential causes underlying higher prevalence of keratoconus could be due to a host of factors including geographic locations, ultraviolet irradiation exposure, consanguinity, persistent eye rubbing and atopy. The etiology of keratoconus is complex, involving multi-factorial interactions of genetic, personal, and environmental factors.

Study	Ethnicity	Prevalence	Age at diagnosis	Year of report	Reference
Japan	Japanese	7.6 in 100,000 12 in 100,000 males 5.6 in 100,000 females		2002	[52]
Singapore	Malay Indian Chinese	Steep cornea/keratonconus 606 in 100,000 1,000 in 100,000 500 in 100,000		2014	[54]
China	Chinese Bejing Eye Study, northern China	Steep cornea/keratonconus 950 in 100,000	aged 50–93 years	2012	[53]
India	Indian	2300 in 100,000 5200 in 100,000	53.2±11.3 years 21.5 years	2015,2009	[50], [51]
Iran		25 per 100,000 4000 in 100,000	27.1±9.3 years	2012,2018	[46], [47]
Saudi Arabia	Saudi Arabian	20 in 100,000	17.7 ± 3.6 years for males (range 8–24 years) 19.0±3.8 years for females (range 12–28 years)	2005	[48]
U.K.	Caucasians Indians	57 in 100,000 229 in 100,000	26.5 years 22.3 years	2000	[43]
U.S.A.	Whites and Blacks Whites, Blacks and Hispanics	54 in 100,000 17.5/100,000		1986 2009	[3], [41]

Table 1.
Geographical and ethnical diversities in reported prevalence of keratoconus.

5. Gender differences

Whether males and females have different prevalence is unclear as inconsistent results have been reported [55]. Disease onset in males tend to be earlier and disease progression faster than female patients in both Asian and Western studies, while gender bias has not been consistent [40, 50, 56, 57]. Male and female sex, did not show difference in prevalence, while gender bias have not been consistent in previous reports. In a Japanese cohort of 90 patients, men were diagnosed younger than women [58]. A questionnaire survey of 670 patients in New Zealand also showed male patients were detected at younger ages than females [59]. In a Turkish cohort of 248 patients, there was no gender difference in cornea properties including central cornea thickness and keratometry parameters [56]. In a study in the USA of 1209 patients from 16 clinics, while there was no difference in disease severity according to keratometry or scarring, less women were had have Vogt striae [57]. Female patients in this study had higher mean age than the males. Overall, there was indication that men developed keratoconus earlier, progressed faster and required more serious treatment.

6. Twins and familial segregation

6.1 Twins with keratoconus

As early as 1954 occurrence of keratoconus in both identical twins had been reported [60]. Following twins reports in different ethnic groups, one twin pair was found to show different contrast sensitivities [61]. However, two pairs of Caucasian identical twins both showed similar features clinically and under videokeratography and radial keratotomy, as one pair were at early and the other as later stage [62]. Two pairs of monozygotic twins were found discordant for keratoconus in the USA, one from a hispanic family of Mexican descent and the other Caucasian from England [63], while dissimilarity in phenotype may suggest the absence of genetic involvement. However, natural monozygotic discordance could occur if there was separation of the zygote into two distinct cell masses after fertilization before the start of tissue differentiation. Post-zygotic events that lead to existence of two different cell clones in the early zygote may precede the twinning process. In the Mexican family, 39 members were examined and 5 were suspected for keratoconus by corneal topography. Also, one distant relative was a confirmed keratoconus patient. Corneal topography also revealed one suspect from 59 family members examined in the English family. There could be a genetic component in the keratoconus phenotype in these two families [63]. Of note, more and more concordant twins with keratoconus have been reported, including reported concordance in all 13 monozygotic and 5 dizygotic twins [64].

6.2 Familial linkage

In the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) study in a cohort of 1209 patients mixed in ethnicity in the USA, 829 (69%) white, 240 (20%) black, and 99 (8%) Hispanic, family history of keratoconus was reported in 13.5% of the cases [65]. After follow-up for 8 years, the inheritance patterns were not established [66]. In another study from the USA, more females reported family history than males. But it was unclear whether it was a difference in attitude on reporting or a genuine gender difference in familial link [57]. In the New Zealand study, familial aggregation analysis showed keratoconus familial rate of 23.5% [59]. In Scotland, family history occurred in 5% of 186 white patients [67]. In a report from North India among 120 patients, 6 (5%) had family history [68]. In a review of keratoconus

in Asians, family history ranged from 4.4 to 23.5% [40]. Overall, reported family history of keratoconus has widely ranged from 6% up to about 25% [37, 65].

A recent systemic review and meta-analysis on 29 eligible reports from different parts of the world selected from 3996 articles revealed family history as the strongest risk factor (odds ratio 6.42; 95% CI:2.59–10.24) among other established risk factors: eye rubbing (odds ratio 3.09; 95% CI:2.17–4.00), eczema (odds ratio 2.95; 95% CI:1.30–4.59), asthma (odds ratio 1.94; 95% CI:1.30–2.58), and allergy (odds ratio 1.42; 95% CI:1.06–1.79) [69]. Overall, no obvious differences exist in clinical or ophthalmic presentations between familial and sporadic keratoconus. As a genetic disease, keratoconus has shown weak penetrance with variable expressions. While family aggregation and linkage studies showed familial inheritance, the majority of reported keratoconus patients are sporadic.

7. Mapping the keratoconus genes

Candidate gene analysis, family linkage analysis, and more recently genome-wide association study with support by candidate gene association studies and next generation sequencing, have contributed to identification of genetic loci or gene variants that are in association with keratoconus [70]. All are genetic associations. No direct keratoconus causing mutation has been identified.

7.1 Linkage analysis

Single nucleotide polymorphisms (SNPs) and microsatellite markers covering the whole genome have been attempted to find keratoconus loci or even genes in family pedigrees and sib pairs. But even in families, penetrance of keratoconus is low and clinical presentations are variable. A large number of samples have to be available. Vigorous research among various ethnic groups have identified a number of keratoconus loci which are replicable and of maximum LOD score greater than 3, in a collection of 67 sib pair Hispanic families, two-stage genome-wide analysis of 380 microsatellite markers in totally 351 subjects, among them 110 were affected by keratoconus which has led to identification of a multitude of loci in chromosomes *2q, 3p, 4q, 5q31, 5p, 9p, 9q34, 11p, 12p, 14q*, and *17q* [71]. In a collection of 25 Italian families, genome-wide scan of microsatellite markers in 77 affected and 57 unaffected members have mapped chromosomal regions for keratoconus in *5q32-q33; 5q21.2, 14q11.2,* and *15q2.32* [72]. Some of these loci had been replicated or refined in further investigations: *2p24* [73]; *3p14-q13* [74]; *5q14.3-q21.1* [75]; 5q31.1-q35.3 [72]; *13q32* [76]; *16q22.3-q23.1* [77]; and *20q12* [78]. These studies were mostly on European populations. The large number of associated loci exemplified the genetic heterogeneity of keratoconus. No disease-causing mutation has been identified from these loci.

7.2 Genome-wide association studies (GWAS)

Most GWAS in connection with keratoconus were conducted on the two quantitative traits of central corneal thickness (CCT) and corneal curvature (CC). These two are characteristic, but not exclusive, traits of keratoconus, with the corresponding morphological features of central corneal thinning and corneal steepness.

7.2.1 Central corneal thickness (CCT)

There were nine reported GWAS on central corneal thickness (CCT) up to July 2017. They were summarized in an excellent review [70]. All involved meta-analysis

in initial and validation cohorts. More than 40 SNPs in 30 genes were reported. The biggest sample size was with totally 13,057 European and 6963 Asian samples, while the primary cohort was comprised of 874 patients and 6085 controls. A multitude of keratoconus associated SNPs in 26 loci was identified. SNP rs9938149 in the *BANP-ZNF469* gene attained GWAS significance of P-value = 2.4×10^{-49}, which was the highest among all reported SNPs [79].

Some of the gene variants were analyzed in keratoconus and control cohorts. Possibly there was ethnic specificity. Rs96067 in COL8A2 was identified in a Singaporean cohort of 2538 Indians and 2542 Malays [80] but not in a separate GWAS study 0f European study subjects with 3931 German and 1418 Dutch study subjects. However, SNPs in *BANP-ZNF469* and COL5A1 were replicated [81]. In a cohort of Australian white study subjects with 933 keratoconus patients and more than 4000 controls, keratoconus susceptibility was detected at the *HGF* locus [82]. The risk factor allele rs3735520 was associated with keratoconus in a Czech cohort of 165 patients and 193 controls [83] and Australian whites of 157 patients and 673 controls [84]. Another study involved two independent cohorts of keratoconus patients, involving 222 Caucasian patients, 687 African Americans, 3324 Caucasian controls and 307 individuals from 70 keratoconus families reported strong association of *Lysyl Oxidase* gene (*LOX)* polymorphisms with keratoconus, with meta P-values of 2.5×10^{-7} and 4.0×10^{-5} for LOX SNPs rs2956540 and rs10519694 respectively [85]. In a meta-analysis of 14 studies comprising 17,803 individuals of European ancestry 44 loci associated with CCT were found, two of them were LTBP1 and WNT10A 42 European specific while the rest occurred also in Asian ethnicities [86].

7.2.2 Corneal curvature (CC)

Six GWAS on corneal curvature (CC) have been reported in multi-ethnic cohorts that contributed to identification of susceptibility genes. Eight SNPs in MTOR/FRAP1 and 7 SNPs in PDGFRA were found in 10,008 samples from three population groups in Singapore: Malays, Chinese and Indians [87]. Another big cohort of 12,660 Asians included Japanese in addition to Malays, Chinese, and Indians, [88] Associations of CMPK1 rs17103186 and RBP3 rs11204213 with CC, and RBP3 rs11204213 with axial length were discovered. In the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort of 2023 individuals of white European descent, rs6554163 in *PDGFRA* was associated with both CC and axial length [89]. In a cohort of 1013 Australian individuals, 1788 twins and their families, CC was associate with rs2114039 near *PDGFRA* and rs2444240 which is 31 kb upstream to *TRIM29*, but not with any SNP at the FRAP1 locus [90]. In totally 15,168 study samples that included Japanese, Chinese or European ethicity, rs10453441 in WNT7B was strongly associated with both CC and axial length [91]. WNT7B rs10453441 is the only SNP associated with both traits of CCT and CC [92]. While these SNPs have no consistent and strong association with keratoconus, an exome sequencing analysis identified a *WNT10A* variant that was associated with corneal thickness and keratoconus [93]. In contrast, a GWAS on direct association of keratoconus patients and controls involving 222 patients and 3324 controls found no GWAS significance of associated gene variants [94].

7.2.3 Other approaches

Apart from corneal morphologic features, a recent GWAS investigated corneal biomechanical properties in an European cohort of 6645 participants and 2384 participants from the British TwinsUK study [36]. The second stage of the association

study involved 752 keratoconus patients as compared with 974 British TwinsUK or 13,828 EPIC-Norfolk. The results showed a likely role in development of keratoconus with 5 associated loci in CH, *ANAPC1*, *ADAMTS8*, *ADAMTS17*, *ABCA6*, and *COL6A1* [36].

It is notable that there are a lot more keratoconus genes that are identified through studies on quantitative traits, especially central cornea thickness and cornea curvature, than on direct association with keratoconus against controls (**Figure 1**).

7.3 Candidate genes

Strategies of genomic search for disease genes are essentially free of a hypothesis to find genes or loci with susceptibility to a disease entity or quantitative trait. For keratoconus, mutation analysis in many candidate genes have also been attempted to find disease causative genes, some followed a biological hypothesis and some based on high GWAS significance. More than 50 SNPs in about 20 genes showing association with keratoconus have been studied in various ethnic populations [70, 95]. They include *FOXO1* [96]; ZNF469 [97]; *COL4A4* [98]; *COL4A3* [99]; *VSX*1 [100, 101]; *COL5A1* [102]; *MPDZ-NFIB* [102, 103]; *IL1B* [104, 105]; *HGF* [84]; *LOX* [85, 106]; and *IL1RN* [107]. Some of these genes have been studied in many populations with inconsistent results.

7.3.1 VSX1

The *VSX1* (visual system homeobox 1) gene has been regarded as a candidate keratoconus with about 20 missense variants being putatively disease causative [101, 108]. *VSX1* sequence variants have been extensively screened in different populations including Caucasians, Indians, Chinese, Iranians and Koreans. But segregation of *VSX1* missense variants with keratoconus has been inconsistent and there is no confirmed causative mutation in *VSX1* for keratoncous. p.Leu268His (c.803 T > A)

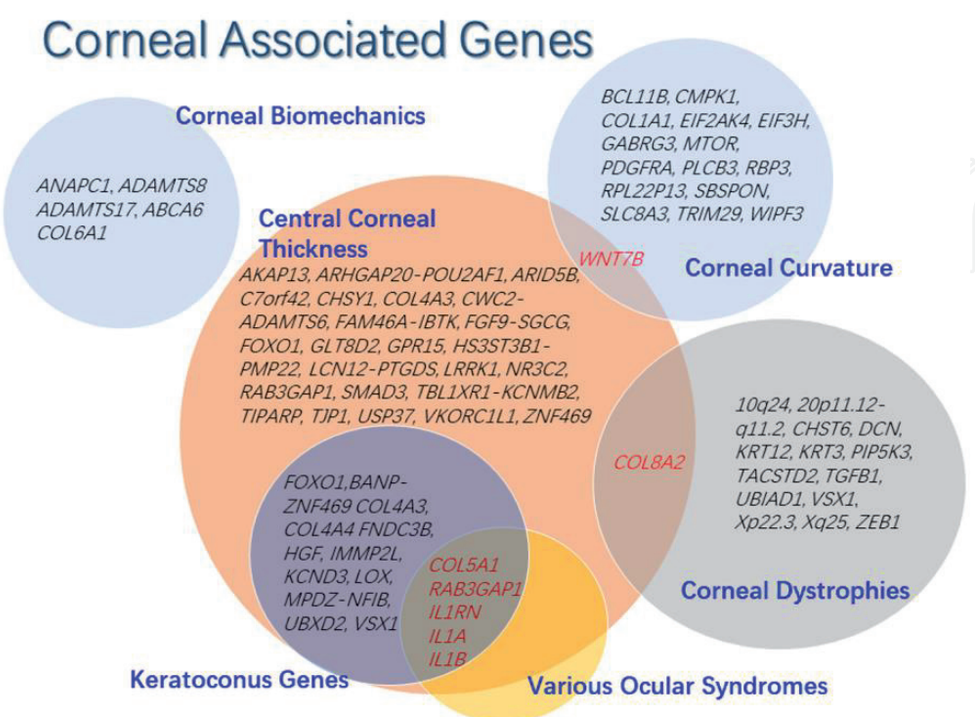

Figure 1.
Cornea associated genes.

was proposed to be foundation mutation as a shared haplotype occurred in 5 Indian keratoconus patients from 2 unrelated families [109]. But it has not been reported in other studies on Indian, Iranian, Korean and other populations.

7.3.2 IL1A and IL1B

The interleukin genes *IL1A* and *IL1B* have been studied in several keratoconus cohorts as they are mediators of keratocyte apoptosis which may occur in corneal injuries that lead to epithelial or endothelial-stromal reorganization as in keratoconus [110, 111]. Screening for *IL1* gene cluster mutations in a Korean cohort of 100 patients and 100 controls identified −31*C (rs1143627) and − 511*T (rs16944) in the *IL1B* promoter posed risk for keratoconus with a combined significance of P = 0.012, OR = 2.38, 95% CI = 1.116-5.05) [104]. Similar association in a Japanese study of 169 patients and 390 controls was reported with a haplotype of −31*C and - 511*T, P = 4.0×10^{-5} and OR = 1.72 [105]. The association was replicated in 115 Han Chinese patients and 101 healthy controls, with significance for −31*C, P > 0.001, OR = 2.86, and P = 0.002, OR = 2.4 for −511*T. SNP IL1A rs2071376 also showed association with P = 0.017, OR = 1.97. The respective ACA haplotype of these 3 promoter SNPs was found to contribute a high risk in this Chinese cohort, P < 0.001, OR = 12.91 [112]. Such statistical significance shows a link of *IL1A* and *IL1B* with keratoconus, and the reported associations are more consistent than other candidate genes. It should be of interest to study the biological effects of these promoter polymorphisms on corneal tissue cells.

7.3.3 MPDZ-NF1B

The *MPDZ* (multiple PDZ domain crumbs cell polarity complex component) and *NFIB* (nuclear factor I B *NF1B*) genes was found to confer risk to keratoconus based on GWAS on central corneal thickness (CCT) of multi-ethnic Asian populations in Singapore. rs1324183 of *MPDZ-NF1B* gave a significance of $P = 8.72 \times 10^{-8}$ [113]. The association was replicated inn Australian Caucasian cohort, P = 0.001, OR = 1 [114]. Further examination of the CCT loci in keratoconus patients from two independent Caucasian cohorts also revealed association rs1324183 for keratoconus, $P = 5.2 \times 10^{-6}$, OR = 1.33 [79]. In a Han Chinese cohort of 210 patients and 191 controls in northern China, the association was p = 0.005, OR = 3.1 [115]. However, no association was found in a Saudi Arabia study of 108 patients and 300 controls [116]. In contrast, very high risk of rs1324183 to keratoconus, $P = 4.30 \times 10^{-4}$ OR = 2.22 was shown after genotyping of 133 patients and 367 controls who are Han Chinese in Hong Kong in southern China [117]. In addition, rs1324183 conferred a higher risk to severe keratoconus (OR = 5.10) than the moderate (OR = 2.05) or mild (OR 2.36) form. There was significant correlation between the risk allele A of rs1324183 with the corneal mechanic parameters of anterior Kf, anterior AvgK, posterior Kf and apex pachymetry, indicating association with corneal thickness and curvature. Therefore rs1324183 has been proposed to be a genetic marker for severity and progression of keratoconus [117]. Taken together, there is no evidence of direct causation to keratoconus by *MPDZ-NF1B,* which, however, does pose susceptibility to development and progression of keratoconus.

7.3.4 COL4A3, COL4A4 and COL5A1

The collagen genes *COL4A3*, *COL4A4* and *COL5A1* are related to corneal collagen structure and development during embryonic development. Its association with keratoconus was first reported in a Slovenia study of white study subjects,

104 patients and 157 controls, with 3 variants, P141L, D326Y, and G895G, in COL4A3 and 5 variants in COL4A4P482S, M1327 V, V1516 V, and F1644F differentiating patients and controls with statistical significance $P < 0.005$ [118]. Association was replicated in a Greek study [99]. In an Iranian cohort of 112 patients and 150 controls, The COL4A4 rs2229813 (M1327 V) An allele increased keratoconus risk for KC ($P = 0.018$, OR = 1.5), but COL4A4 rs2228555 (C1516V) showed no association [98].

Another COL4A4 SNP, rs2228557 (F1644F) in 144 patients, 153 controls in Iran, showed a high association ($P = 0.0001$) [119]. *COL4A4* rs2229813 and rs2228557 are strongly associated with keratonconus in Americans ($P = 1.3 \times 10^{-12}$, OR = 2.38 and $P = 4.5 \times 10^{-7}$, OR = 0.54 respectively) [102]. Replication in a Chinese cohort showed weak association [120]. Although *COL4A3* and *COL4A4* plays biological roles in cornea structure and properties, there is no evidence that they directly cause keratoconus.

8. Specific proteins

The pathophysiology of keratoconus is attributed to disruption of the cornea morphology in association with the corneal collagen. A review of published studies on proteins, collagen and risk factors of keratoconus between January 2016 and June 2018 has revealed altered regulations in more than 30 proteins and their genes. They belong to protein families including degradative enzymes, cellular proteases, and collagens [121]. Up- and downregulations of hosts of proteins in corneal epithelium and stroma have been reported in keratoconus, especially different types of collagen and matrix metalloproteinases.

8.1 Collagen

Collagen is the major structural protein in the cornea. Decrease in collagen lamellae and fibers, together with reduced microfibers and fine granules, has been described in keratoconus [122]. Disruption in collagen contents and integrity affect corneal thinning and properties. Reduction of types I, III, and IV, which are major structural proteins in the basement membranes, have been shown in keratoconus [123]. Among the 6 main subtypes of COL4A, which plays important structural and functional roles, COLA4A1 is reportedly downregulated in cornea of keratoconus patients [124]. Expression studies also showed downregulation of many collagen types and subtypes in keratoconus, including COL8A1, COL8A2, COL12A1, COL13A1, COL6A2, COL15A1, and COL18A1 [125–127]. They may be considered for use as biomarkers in keratoconus, [121] but practical protocols and validations are still to be established.

8.2 Matrix metalloproteinases

MMP-1 and MMP-9, are upregulated in corneal tissue and affect collagen properites and dyregulate proteolysis [128]. In a pathways enrichment analysis of 19 keratoconus genes consistently reported as risk genes to keratoconus in 16 studies, interleukin-1 processing and assembly of collagen fibrils are associated with keratoconus pathology [129]. MMPs have been assayed in tears. It is noted that in one study there was no detectable MMP-1 in tears of healthy subjects [130]. Elevations in MMP-1 [131] and MMP-9 levels [132] has been shown in keratoconus tear samples. On study reported correlation between MMP-9 level and disease severity [133]. However, other studies did not find MMP-9 elevation [131, 134].

Inconsistent findings are also reported for MMP-3, MMP-7 and MMP-13 [128]. All in all, there are consistent evidence on elevated MMP-9 levels in keratoconus cornea tissues and tears. Given the important role of MMP-9 in extracellular matrix regulations and corneal inflammation, MMP-9 assay should be useful for monitoring keratoconus treatment. A point-of-care test for MMP-9 in tears has been established for quick and reliable MMP-9 tear essay that facilitates the treatment monitoring [135].

9. Molecular markers

After meta-analysis of 24 eligible studies selected after screening of 668 reports between 1950 and 2016, 16 SNPs in 14 genes/loci were found to be associated with keratoconus out of 53 polymorphisms in 28 genes/loci. Stratification analysis revealed strong association of 8 SNPs in 6 genes/loci with keratoconus in Whites, including *FOXO1* rs2721051, *RXRA-COL5A1* rs1536482, *FNDC3B* rs4894535, *IMMP2L* rs757219 and rs214884, and *BANP-ZNF469* rs9938149, and *COL4A4.*

rs2229813 and rs2228557 [95]. While they may be potential genetic markers for keratoconus in Whites, there is no further data of validation. Replications in Chinese and Arabic populations [120] revealed weak associations in *COL4A4* rs2229813 and rs2228557, which are strongly associated with keratonconus in Whites with statistical significance of $P = 1.3 \times 10^{-12}$, OR = 2.38 and $P = 4.5 \times 10^{-7}$, OR = 0.54 respectively. In Chinese, another SNP, rs1324183 in *MPDZ-NF1B*, is related to disease severity and corneal biomechanical properties, and may be a potential molecular marker [136].

In a review in 2001 keratoconus was reputed to be an inheritable disease [137]. Since then more evidences of familial links and susceptible genes or loci have been reported as a result of vigorous research in different populations and geographic locations. There are proven genetic and non-genetic risk factors [35]. In a recent

Genes	Variants	Study design	Study population	Keratoconus patients (n)	Controls (n)	*P*	OR (95%CI)	Reference
HGF and 12 loci	rs3735520 rs17501108 rs1014091	GWAS + Validation	Australia, America	933	4164	0.002 0.0002 0.0004	1.50 (1.15–1.94) 2.33 (1.17–3.69) 2.22 (1.41–3.48)	[82]
3p26,2q21.3,19q13.3 and 12 loci	rs6442925 rs4954218 rs1428642	GWAS + Validation	America	222	3,324	6.5×10−8 2.4×10−7 3.1×10−7	1.85 0.5 0.59	[94]
LOX	rs10519694 rs2956540	GWAS + Validation	America	222	3,324	2.3×10−3 7×10−3	0.67 0.73	[85]
FOXO1 and FNDC3B	rs2721051 rs4894535	GWAS + Validation	Australia, Northern Ireland and America	874	6,085	2.7×10−10 4.9×10−9	1.62 (1.4–1.88) 1.47 (1.29–1.68)	[97]
WNT10A	rs121908120	Exome Sequencing	Australia	621	1,680	6.63×10−10		[93]
TFAM *ND1* *COX1* *ND6* *POLRMT* *TFB2M*		Mitochondrial genome expression study	Chinese	198	106	3.26×10−3 1.79×10−3 1.54×10−3 4.62×10−3 2.55×10−4 7.88×10−5		[138]
VSX1	rs72106541 rs8123716	Sanger sequencing	Polish	42	50	0.491 0.278		[139]
TNFa	rs1800629	Case-control Association Study	Pakistan	257	253	<0.001	6.67 (4.28-10.42)	[140]
MPDZ, *FOXO1,* *MAP3K19/RAB3GAP1*	rs1324183 rs2721051 rs4954218	Case-control Association Study	Czech	165	193	0.01 0.025 0.047	1.58 (1.10-2.24) 1.72 (1.07-2.77) 1.53 (1.01-2.34)	[141]
VSX1	R131P G160V	Case-control Association Study	Chinese	50	50		R131P in 1 patient G160V in 2 patients none in controls	[142]
	L68H rs6115023 (D105E) rs6050307 (R131S)	Exome Sequencing	Brazilian	73	107	0.121 0.108	L68H in 3 patients 3.86 (0.73-20.5) 2.73 (0.77-9.68)	[144]
TUBA3D	Q11X V68Lfs*2 c.*2 G > A	Exome Sequencing	Chinese	202	200		Q11X in 1 twins family V68Lfs*2 in 1 patient c.*2 G > A in 1 patient none in controls	[143]
COL4A3 *MPDZ*		Exome Sequencing and targeted gene sequencing	Australian	385	396	0.024 0.045	0.54 (0.31-0.96) 0.65 (0.42-0.99)	[145]
CAST	rs4434401	Case-control Association Study	Chinese	120	305	0.037	1.47 (1.02-2.11)	[146]

Table 2.
The keratoconus genes [138–146].

comprehensive review of environmental risk factors and family history, genetic factors are taken to play a role in the etiology of keratoconus [39]. As of today all reported genes for keratoconus, whether mapped by candidate gene strategy or genomic search including GWAS and WES, are susceptibility genes and not causative genes that cause disease directly (**Table 2**). Keratoconus causative genes are still to be identified. As for the reported genes, there is no segregation of gene variants that accounts for higher occurrences of disease. There are also no hotspot variants that are present in a high proportion of patients. Molecular markers for pre- symptomatic detection and risk assessment of keratonocus are still to be established.

10. Genetic implications on treatment

Findings in genetic studies help to delineate the molecular basis of diseases through identification of genes that are causative or susceptible to development of diseases. Investigation of their properties, functions, related pathways and mechanisms throw light on disease pathogenesis. Genetic information also helps to establish genetic markers used for early or even pre-symptomatic diagnosis. Prior to treatment timely detection is extremely important as keratoconus is progressive and the resultant corneal disruptions are hardly curable. With the advent of collagen cross-linking, disease progression can be halted in most patients with some partial recovery of vision. Some patients may respond less favorably and ultimately may require cornea transplantation. Genetic marker, if linked to response to clinical course and treatment, will be exceedingly useful. Over the years in keratoconus vigorous research has been conducted in different ethnic populations in its molecular genetics. However, with the repertoire of associated genes that has been identified at present, no definite genetic marker for diagnosis, risk assessment or prognosis has been established. Further work is warranted.

11. Conclusive remarks and future perspectives

The pathogenesis of keratoconus is heterogeneous and complex. Epidemiological studies showed higher prevalence, earlier onset and greater progression in Asians than Europeans. Both environmental and genetic factors play roles in the etiology and pathogenesis, including age, gender, ocular atopy, eye rubbing, family history, and systemic diseases. While family aggregation and linkage studies indicated genetic abnormality in keratoconus, GWAS and candidate gene studies identified polymorphisms in genes/loci related to the risk of keratoconus. So far there are very few reported big family studies, which should help to identify the keratoconus causative gene. Also, big cohorts are needed to provide sufficient power to differentiate phenotypes and clinical courses of patients for association with genetic factors. Current epidemiological and genetic data are insufficient to provide conclusive evidence to establish the molecular mechanism and genetic markers for keratoconus. Notably, genetic studies on the corneal structure, principally central cornea thickness and cornea curvature have successfully mapped keratoconus genes. Corneal properties, as recently exemplified by a successful GWAS on corneal biomechanical properties [36], should provide a basis for genetic research. Rigorous and large multi-center population-based studies, with age-standardized rates, random sampling, progression follow-ups, and accurate and standardized diagnosis, are warranted for better understanding of pathogenesis of keratoconus and for establishment of genetic markers.

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