



The genetics of keratoconus

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Keratoconus is a heterogeneous disorder. To evaluate critically whether keratoconus is a genetic disorder and what contribution genes make to its pathogenesis, keratoconus should be divided into three broad categories:

1. Isolated keratoconus associated with rare genetic disorders (Box 1)
2. Keratoconus in the setting of commonly reported associations (Box 2)
3. Isolated keratoconus with no associations

Isolated keratoconus with no associations is by far the most common presentation seen by a practicing clinician [1]. Categories 1 and 2 account for less than 2% of 1200 consecutive keratoconus patients seen at the Keratoconus Genetics Research Program at Cedars-Sinai Medical Center. The genetics of the most common form of keratoconus (isolated keratoconus with no known associations) are explored in this article.

Isolated keratoconus is itself heterogeneous and has been reported in several settings which include eye rubbing [2], allergy [3,4], connective tissue dysfunction [5–7], contact lens wear [8], and in a familial setting [9–12]. Before commencing formal genetic analyses of keratoconus patients, the author and colleagues used a carefully designed questionnaire to

compare possible causative factors in 218 keratoconus patients and in 183 normal age-matched controls. The factors included contact lens wear, allergy, joint hypermobility, eye rubbing, and family history. Table 1 summarizes the results of these analyses. The only two factors for which statistically significant differences between the groups were observed were eye rubbing and family history. Eighty-three percent of keratoconus patients admitted to rubbing their eyes frequently, but so did 58% of normal controls. Ten percent of the keratoconus patients had a positive family history for keratoconus, versus only 0.5% of the age-matched controls [13].

There are several indications that genetic factors play a role in the pathogenesis of isolated keratoconus. This evidence arises from twin studies [14–25], the bilaterality of the disorder [26–28], reports of familial aggregation [29,31], and formal genetic analyses [32]. Twins have a special place in human genetics because of their usefulness for comparison of the effects of genes and environment. (The importance of twin studies for comparison of the effects of nature and nurture was originally pointed out by Galton in 1875 [33]). Diseases caused wholly or partly by genetic factors have a higher concordance rate in monozygotic twins than in dizygotic twins. Even if a condition does not show a simple genetic pattern, comparison of its incidence in monozygotic and dizygotic twin pairs can reveal that heredity is involved; moreover, if monozygotic twins are not fully concordant for a given condition, nongenetic factors must also play a part in its etiology. In the published literature, there are at least 18 sets of monozygotic twins in which one or both twins show some degree of keratoconus. Thirteen of these pairs were described before the advent or without the use of videokeratography: Seven pairs were con-

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Box 1. Diseases reported in association with keratoconus

Multisystem

Alagille syndrome
 Albers-Schonberg syndrome
 Angleman's syndrome
 Apert's syndrome
 Autographism
 Anetoderma
 Bardet-Biedl syndrome
 Crouzon's syndrome
 Down syndrome
 Ehlers-Danlos syndrome
 Goltz-Gorlin syndrome
 Hyperornithemia
 Ichthyosis
 Kurz syndrome
 Laurence-Moon-Biedl syndrome
 Marfan syndrome
 Mulvihill-Smith syndrome
 Nail-patella syndrome
 Neurocutaneous angiomatosis
 Neurofibromatosis
 Noonan's syndrome
 Osteogenesis imperfecta
 Oculodentodigital syndrome
 Pseudoxanthoma elasticum
 Rieger's syndrome
 Rothmund's syndrome
 Tourette's syndrome
 Turner's syndrome
 Xeroderma pigmentosa

Ocular

Aniridia
 Ankyloblepharon
 Bilateral macular coloboma
 Blue sclerae
 Congenital cataracts
 Ectodermal and mesodermal anomalies
 Floppy eyelid syndrome
 Gyrate atrophy
 Iridoschisis
 Leber's congenital amaurosis
 Anetoderma and bilateral subcapsular cataracts
 Microcornea
 Persistent pupillary membrane
 Posterior lenticonus

Retinitis pigmentosa
 Retinopathy of prematurity
 Retrolental fibroplasia
 Vernal conjunctivitis

Corneal

Atopic keratoconjunctivitis
 Axenfeld's anomaly
 Avellino dystrophy
 Chandler's syndrome
 Corneal amyloidosis
 Deep filiform corneal dystrophy
 Essential iris atrophy
 Fleck corneal dystrophy
 Fuch's corneal dystrophy
 Iridocorneal dysgenesis
 Lattice dystrophy
 Pellucid marginal degeneration
 Posterior polymorphous dystrophy
 Terrien's marginal degeneration

Other

Congenital hip dysplasia
 False chordae tendinae of left ventricle
 Joint hypermobility
 Mitral valve prolapse
 Measles retinopathy
 Ocular hypertension
 Thalesseis syndrome

cordant, and six pairs were discordant for keratoconus [34]. Because of topography and other confounding factors (eg, assessment at too young an age), these 13 cases, and in particular the six discordant pairs, cannot be considered conclusive. Of greater interest are the monozygotic twins examined with videokeratography. Bechara et al [23] described two sets concordant for keratoconus. Owens and Watters [22] described

Box 2. Clinical settings in which isolated keratoconus may occur

Contact lens wear
 Eye rubbing
 Atopy
 Down syndrome
 Leber's congenital amaurosis
 Mitral valve prolapse
 Positive family history

Table 1
Etiologic factors (response to questionnaire)

	Normal Controls (N = 183)	Keratoconus Patients (N = 218)
Allergy	66 (35%)	96 (44%) $P < 0.105$ (NS)
Joint hypermobility	10 (12%)	34 (15%) $P < 0.305$ (NS)
Eye rubbing	106 (58%)	174 (80%) $P = 0.001$
Positive family history	1 (.05%)	22 (10%) $P = 0.001$

Abbreviation: NS, not significant.

two pairs in which both twins had the condition but were topographically discordant; Parker [24] reported a similar case. McMahon et al [25] reported two sets of proven monozygotic twins in which one twin had clinical keratoconus and the other twin had normal videokeratography.

Reports in the literature therefore report monozygotic twins concordant rather than discordant for keratoconus. This finding strongly supports a genetic origin for the disease. The cases of proven monozygotic discordance may indicate that an environmental cofactor, in addition to a genetic susceptibility, is necessary for clinical manifestation of the disease and might also explain topographic variation between a pair of monozygotic twins. A primary genetic cause in discordant twins is still possible in light of these data, however, because recent studies suggest that monozygotic twins may not be genetically identical in all tissues [34]. There are few data on dizygotic twins with keratoconus. Documented reduced concordance in comparison to the rate of monozygotic twins would be further evidence suggesting genetic influences. Although most reports in the literature on twins strongly support a genetic origin for keratoconus, a formal, prospective twin study comparing monozygotic versus dizygotic twins without ascertainment bias would strengthen this argument.

Corneal dystrophies, all of which have a genetic basis, are almost universally bilateral. Most patients with keratoconus have bilateral disease. (In many instances the disease may initially be unilateral, but over time the contralateral eye becomes involved, although the involvement of the contralateral eye may be subclinical.) Many reports in the literature document the bilaterality of the disorder; reports by Holland et al [28], Lee et al [27], and Wilson all suggest that if videokeratography is used to study both eyes, more than 90% of patients with keratoconus will exhibit evidence for bilateral disease. The bilateral nature of keratoconus, similar to that of corneal dystrophies, strongly suggests that ikeratoconus has a genetic basis.

Familial aggregation of a disease is one of the most commonly recognized indications of genetic influ-

ences in a disease. Although keratoconus is most commonly reported as sporadic, the incidence of familial aggregation reported in the literature ranges from 6% to 23.5% [13,14,29,30,35,36]. In studies at Cedars-Sinai Medical Center, 10% of study subjects had a positive family history for keratoconus. It could be argued that there may be a selection bias in these studies that recruit patients specifically for genetic studies, because patients with a family history would naturally gravitate to the study. The Collaborative Longitudinal Evaluation of Keratoconus Study, however, reported a positive family history of 14%. This study was a multicenter, longitudinal study to observe the natural history of keratoconus without the ascertainment bias that could be ascribed to genetic studies [29]. Both these studies are from a broad, diverse, and ethnically heterogeneous group of patients. In ethnically more homogenous populations, such as those certain parts of Finland, New Zealand, and Tasmania, the incidence of familial disease is much higher, in the order of 19% to 23%. More pronounced gene pooling from larger families with common ancestry is, however, implicated in these unusual populations [14,36]. Most studies on familiarity in all probability underreport the true incidence of a genetic history, because subtle degrees of keratoconus can remain undetected in family members who may not be aware that they have the disease. Most reports in the literature suggest an autosomal dominant mode of inheritance with variable expression, with emphasis being placed on subtle forms of the disorder, such as keratoconus fruste or mild irregular astigmatism. Multiple instances have been reported in the ophthalmic literature. Falls [12] cited 21 cases, including one of his own. In a Finnish study, Ihalainen [14] examined 24 cases. In 10 of the 52 families (19%) examined by Hammerstien [16], keratoconus was detected in two or more relatives. The degree of penetrance was approximately 20%. The disease was characterized by complete penetrance and variable expressivity. Redmond [11] reported seven pedigrees. He suggested that keratoconus fruste and high degrees of astigmatism represent incomplete expression of the keratoconus gene and should be

taken into account in pedigree analysis. The author's group used videokeratography to detect abortive forms of the disorder in the families of five patients with keratoconus. In all five families, hereditary patterns were consistent with autosomal dominant transmission with variable expressivity [37]. Gonzalez [38] detected videokeratographic abnormalities in at least one parent in the seven sets of clinically normal parents of patients with keratoconus. Several reports suggest recessive inheritance [39], but in none of these reports is there clear evidence that three generations were examined or that subtle forms of the disorder were included in the pedigree analysis. For the most part it is likely that familial aggregation has been underreported because subclinical forms of keratoconus detectable only by videokeratography have not been included in pedigree analyses [1,40,41].

The strongest evidence for a genetic basis for keratoconus to date are the formal genetic studies (segregation analysis) performed at Cedars-Sinai Medical Center on 95 keratoconus families recruited as part of the Keratoconus Genetics Research program. This study by Wang et al [32] demonstrated that the keratoconus prevalence in first-degree relatives was 3.34%, 15 to 67 times higher than in the general population. It also demonstrated that the correlation of topographic indices was significantly higher in sibling and parent-offspring pairs than in marital pairs, whereas the correlation in marital pairs was no greater than in the normal population. Segregation analyses using clinical criteria and topographic indices (Table 2) rejected both sporadic and environmental models and therefore suggests that genes play a major role in the development of keratoconus and its subclinical indices [32]. Because this study depended the detection of forms of keratoconus by videokeratography indices, it is important to discuss the role of videokeratography in studying and understanding the genetics of keratoconus. This discussion will make the methods, results, and implications of the formal genetic analyses more

meaningful and will provide a better understanding of the genetic basis for keratoconus.

The role of videokeratography studying the genetics of keratoconus

Several reports in the literature suggest that, to understand the genetics of keratoconus and its modes of heredity better, subtle forms of the disorder detectable only by corneal topography should be included in family pedigree analysis [1,40,41].

Marc Amsler [41] using a photographic placido disk, was the first to describe early corneal topographic changes in keratoconus patients before the detection of clinical or biomicroscopic signs. His classic studies on the natural history of keratoconus documented its progression from minor corneal surface distortions to clinically detectable keratoconus. He classified keratoconus into clinically recognizable stages and an earlier latent stage recognizable only by placido disk examination of corneal topography. These early stages were subdivided into two categories: a 1° to 4° deviation of the horizontal axis of the placido disk, which he called keratoconus fruste, and a 4° to 8° deviation of the horizontal axis, which he labeled early or mild keratoconus. Only slight degrees of asymmetric oblique astigmatism could be detected in these early forms of keratoconus. Similar findings were absent in patients with regular astigmatism [41,42].

In Amsler's study of 600 patients [41], 22% had clinically obvious keratoconus in both eyes, 26% had clinical keratoconus in one eye and latent keratoconus in the other, and 52% had latent keratoconus bilaterally. Progression was highly variable and most often asymmetric. The cone could remain stationary, progress rapidly over 3 to 5 years, and arrest or progress intermittently over an extended period of time. When Amsler re-examined 286 eyes 3 to 8 years after the

Table 2
Segregation analysis results

	Using Clinical Criteria Only	Using Topographic Indices
	Keratoconus Patients (N = 218)	Normal Controls (N = 183)
Rejected	No major gene $P < 0.05$	$P < 0.001$
	Sporadic $P < 0.05$	$P < 0.005$
	Environment $P < 0.025$	$P < 0.005$
	Additive $P < 0.05$	$P < 0.010$
	Dominant $P < 0.05$	–
Not rejected	Major gene $P > 0.25$	$P > 0.10$
	Recessive $P > 0.50$	$P > 0.90$
	Dominant –	$P > 0.90$

diagnosis, only 20% of the entire group, including 66% of the latent cases, had progressed. Progression was most likely to occur in patients between 10 and 20 years of age, decreased slightly between the ages of 20 and 30 years, and was less likely to increase after age 30 years [40,41].

The introduction of computer-assisted videokeratometry has provided an opportunity to simulate the work initially proposed by Amlser in a more quantifiable and scientifically reproducible manner. Whereas placido disks provide reasonable coverage of the peripheral cornea, coverage is not absolute, and interpretation of the mires is qualitative, subject to individual observer interpretation, and difficult to quantify. Modern videokeratoscopes provide broad coverage of both the central and peripheral cornea, generating easily interpretable color-coded maps from thousands of data points. Additionally, data can be transformed into indices that are quantifiable and reproducible. These attributes, which allow the recording of curvature changes in both the central and paracentral cornea, make videokeratometry ideally suited for detecting

subtle topographic changes in early keratoconus and for documenting progression by serial topographic analysis (Fig. 1).

Several studies have been performed to characterize the topographic phenotype of clinically detectable keratoconus by videokeratography [40,42]. Most patients have peripheral cones, with steepening extending into the periphery towards the limbus. The steepening in this group is usually confined to one or two quadrants. A smaller group of patients has central topographic alterations. Many central cones have a bow-tie configuration similar to that found in naturally occurring astigmatism. In the keratoconus patients, however, the bow-tie pattern is asymmetric, with the inferior loop being larger in most cases. In contrast to patients with astigmatism, in keratoconus the steep radial axes above and below the horizontal meridian appear skewed, giving the bow tie a lazy-8 configuration. Another pattern found in central cones is more symmetric steepening without a bow-tie appearance. The pattern is usually the same in both eyes of an individual patient, although it may be more advanced

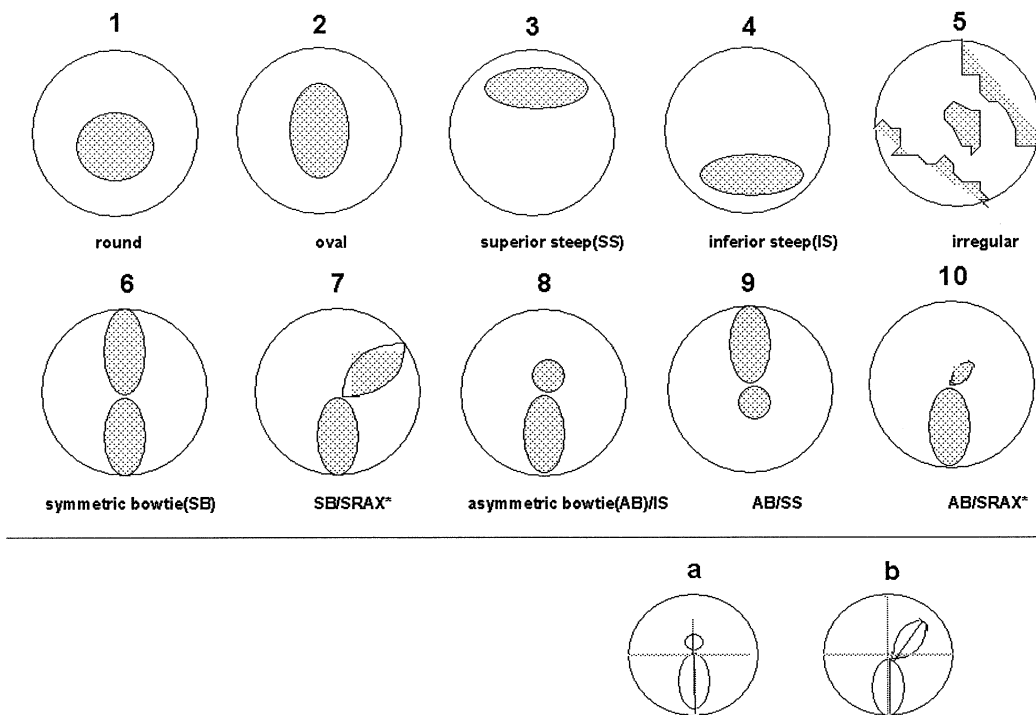


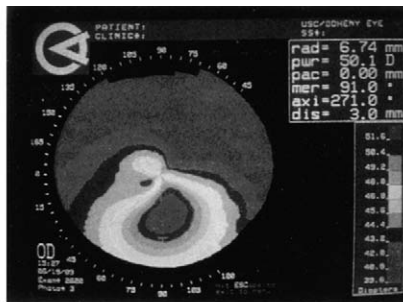
Fig. 1. Classification scheme for normal corneal topography. This pattern suggests that there is a skewing of the steepest radial axes above and below the horizontal meridian. (a and b) An imaginary line is drawn to bisect the upper and lower lobes of the asymmetric bow-tie configuration. (a) If there is no deviation from the vertical meridian, there is no skewing, and the pattern is labeled AB. (b) If the lines bisecting the two lobes are skewed by more than 30° from the vertical meridian, the pattern is labeled AB/SRAX.

in one eye than in the other. The peripheral and central cones probably correspond roughly to the oval sagging and nipple-shaped cones described by Perry [43]. In summary, keratoconus videokeratography has three typical characteristics: an increased area of corneal power surrounded by concentric areas of decreasing power, inferior–superior power asymmetry (I-S value), and skewing of the steepest radial axes above and below the horizontal meridian (AB/SRAX pattern) (Fig. 2) (Box 3).

Similar patterns have been noted in clinically normal family members of keratoconus patients and in the clinically normal eye of patients with clinically unilateral keratoconus [26–28]. These patterns, however, are much milder (as measured by dioptric power) than the patterns noted in clinically obvious keratoconus. Although pattern recognition with color-coded maps becomes relatively easy once the practitioner has gained experience by observing many topographic maps, determining the presence of the minimal topographic criteria required for a diagnosis of keratoconus, based on pattern recognition of a videokeratograph alone, presents a significant difficulty for most clinicians gaining experience with videokeratoscopy. It therefore has been recommended that maps that seem suspicious for keratoconus in the presence of a clinically normal eye be labeled “keratoconus suspect” until progression to keratoconus can be documented [44]. The normalized scale in the videokeratograph, which divides the cornea into 11 equal areas identified by different colors, is confusing, and

many clinically normal patients with slight inferior steepening might inadvertently be labeled as suspect using this scale. One way to become expert at recognizing keratoconus-suspect patterns is to print maps of all patients being examined in the absolute scale (in the TMS-1 (Tomey Technology, Boston, Massachusetts) videokeratograph this scale divides the cornea into 1.5-D intervals between 35 and 50D and into 5-D intervals outside this range) [45]. This singular scale allows the clinician to become familiar with the patterns of a normal eye and recognize abnormal conditions when they are observed. In an attempt to define an early keratoconus phenotype by videokeratography, the author’s group has compiled a database of normal videokeratography patterns of 195 normal individuals using this absolute scale (see Fig. 1). This baseline database of videokeratography patterns (with sagittal topography) was created to be used as a reference for longitudinal topographic studies of keratoconus family members. It can also help the clinician determine whether subtle deviations in corneal topography exist in a particular patient. This database of videokeratography patterns suggests that only 1 of 195 normal patients (0.5%) has mild topographic features similar to those seen in clinically detectable keratoconus (ie, an AB/SRAX pattern) (see Figs. 1–3) [46].

Because clear and consistent definitions of keratoconus are important for accurate genetic pedigree analyses, the author’s group has devised a classification scheme for keratoconus using clinical signs and videokeratography for use in their genetic studies. This



Calculation of I-S Value in Patient 5 (Right Eye)*

Inferior cornea							
degrees	330	300	270	240	210		
diopters	45.9	49.7	50.1	48.6	44.7	239 /5	= 47.8 (I) (average inferior corneal power)
Superior cornea							
degrees	150	120	90	60	30		
diopters	41.6	40.9	40.6	40.2	40.2	203.5/5	= 40.7 (S) (average superior corneal power)

I-S Value = 47.8 (I) – 40.7 (S) = 7.1 diopters

* Measurements taken 3 mm from center of cornea

Fig. 2. Topographic phenotypic features of keratoconus and calculation of the I-S value.

Box 3. Signs of keratoconus*External signs*

- Munson's sign
- Rizzut's phenomenon

Slit-lamp findings

- Stromal thinning
- Posterior stress lines (Vogt's striae)
- Iron ring (Fleischer ring)
- Epithelial or subepithelial scarring

Retroillumination signs

- Scissoring on retinoscopy
- Oil droplet sign ('Charleaux')

Photokeratoscopy signs

- Compression of mires inferotemporally (egg-shaped mires)
- Compression of mires inferiorly or centrally

Videokeratography signs

- Localized increased surface power
- I-S dioptric asymmetry
- AB/SRAX pattern (see Fig. 1)
- Videokeratography indices
 - K value greater than 47.2 (with an AB/SRAX pattern)
 - I-S value greater than 1.6 (with an AB/SRAX pattern)
 - KISA% greater than 100 (The KISA% index is The products of the K value, I-S value, SRAX value, and the regular astigmatism as measured by K readings.)

Fleischer ring, scissoring of the retinoscopic reflex with a fully dilated pupil

An AB/SRAX videokeratography pattern

2. Early keratoconus—no slit-lamp findings but scissoring of the retinoscopic reflex with a fully dilated pupil with an AB/SRAX videokeratography pattern
3. Keratoconus-suspect—no clinical or retroillumination signs of keratoconus but an AB/SRAX videokeratography pattern

These topographic patterns have also now proven useful for constructing family pedigrees for molecular genetic analyses [47].

Videokeratoscopes also have numeric outputs that allow videokeratographic patterns to be quantified independent of subjective pattern analysis. Quantitative descriptors of videokeratographic patterns in keratoconus would allow easier recognition of patterns and enable development of a quantitative phenotype that could be universally used for formulating minimal topographic criteria for diagnosing keratoconus. In a small preliminary study comparing keratoconus and normal videokeratographs, the author's group developed three indices that distinguish keratoconus from normal eyes: central steepening (central K), I-S values (see Fig. 2), and the difference between right and left central corneal power (r versus l). Videokeratography studies on 28 family members of five patients with keratoconus revealed that 50% of the subjects had mild topographic abnormalities and at least one index more than two SD higher than in the normal control group. These abnormalities were similar to but less severe than those found in the patients with keratoconus. This work has been duplicated by several other independent investigators in family studies and in studies of contralateral eyes of keratoconus patients [37,48,49]. These studies suggest that these indices might be descriptive of the earliest stages of keratoconus in normal eyes before they progress to keratoconus.

These indices only quantified two early phenotypic features of keratoconus (ie, central steepening and I-S dioptric asymmetry). The SRAX index that quantifies the irregular astigmatism in keratoconus was also developed to increase the sensitivity and specificity of these descriptors (see Fig. 3). Subsequently, a new single index incorporating all the videokeratographic phenotypic features of keratoconus was developed. [50]. As detailed in Box 4, this index is the product of all previously developed indices descriptive of keratoconus (the K value, I-S value, the SRAX value, and the regular astigmatism as measured by K readings) and is known as the KISA% index.

classification, based on 10 years' observation of the topographic progression of keratoconus-suspect and early keratoconus to clinically significant keratoconus, is as follows:

1. Keratoconus—one or more of the following clinical signs
 - Stromal thinning
 - Vogt's striae

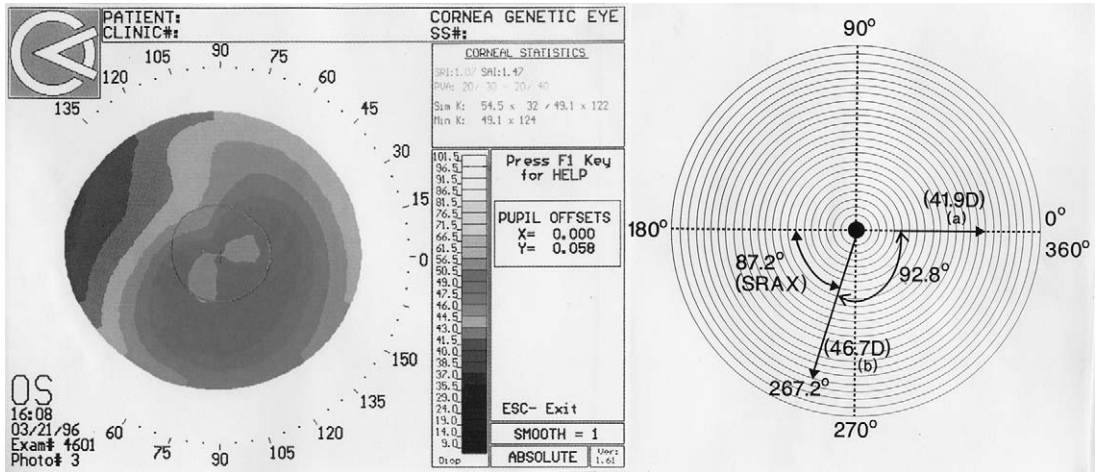


Fig. 3. Topographic phenotypic features of keratoconus and calculation of the SRAX index used in calculating the KISA% index. (Right) Keratoconus videokeratograph (TMS-1 videokeratoscope) demonstrating the three classic phenotypic features of keratoconus (using sagittal topography): central steepening, I-S dioptric asymmetry, and skewing of the steepest radial axes above and below the horizontal meridian. (Left) Calculation of the SRAX index that quantifies the skewing of these radial axes.

This formula produces a % index whereby any patient who has clinical keratoconus has a KISA% index value greater than 100. In a study using this index to study normal controls and clinically obvious keratoconus, at a cutoff point of 100%, 280 of 281 participants (99.6%) were correctly classified (Fig. 4). Six of eight eyes with keratoconus-suspect topography had a KISA% value between 60% and 100%, and 11 of 12 eyes with early keratoconus had a KISA% value greater than 100% [50].

This index has been extremely useful for longitudinal analysis of suspect patterns over time. Fig. 5 illustrates eyes that the author's group has followed for 5 years that have evolved from normal to suspect keratoconus to early keratoconus and then to clinically obvious keratoconus. The index value for each patient has changed as the patterns have changed over time, allowing progression to be quantified in an accurate and reproducible manner.

This single numeric value derived from these indices has significantly advanced in the understanding of the genetics of keratoconus by (1) providing minimal topographic criteria for assigning affection status to keratoconus family members for use in formal pedigree analyses; (2) allowing complex segregation analyses that have demonstrated that genes play a major role in the pathogenesis of keratoconus; and (3) possibly enabling the identification of genes causing keratoconus by using techniques such as genome-

wide screens of multiple families with keratoconus (such studies are currently in progress at Cedars-Sinai Medical Center).

Molecular studies to identify genes causing keratoconus

The genetics of keratoconus is extremely complex and heterogeneous. It is likely that keratoconus is caused by multiple genes and in many instances may result from complex interactions between genes and environmental factors. It is also likely that in different families keratoconus is the result of different gene defects or different gene–environmental interactions. These factors make it difficult to identify clearly a single-gene defect as has been done in other ocular genetic diseases. The best chances of identifying genes are in rare single families or in rare populations with high concentrations of keratoconics because of a common founder. In these situations, heterogeneity is significantly minimized so that results of linkage analysis become more valid.

Although such families are rare, some progress has been made using the linkage approach to identify potential gene loci for keratoconus. To date, only three studies have been performed on families with isolated autosomal dominant keratoconus and no other associated disease. Tyynismaa and coworkers [51] studied

Box 4. Calculation of the KISA% index

The KISA% index quantifies the topographic features seen in patients with clinical keratoconus. It is the product of four indices; the K value (an expression of central corneal steepening); the I-S value; the astigmatism (AST) index that quantifies the degree of regular corneal astigmatism (Sim K-Sim K2); and the SRAX index (an expression of the irregular astigmatism occurring in keratoconus). These individual indices and the methods by which they are calculated have been described in detail elsewhere.

The algorithm for calculating the KISA% index was initially derived as follows:

$$\text{KISA}\% = (\text{K}) \times (\text{I-S}) \times (\text{AST}) \times (\text{SRAX}) \\ \times 100$$

Each individual index quantifies a topographic feature of keratoconus, and any abnormality in one index amplifies the resultant product. The following rules apply:

1. To amplify any abnormality, the value of 1 is substituted in the equation whenever any calculated index has a value of less than 1.
2. Only absolute values are used (eg, an I-S value of -2.0 is corrected to 2.0). Therefore there can be no negative values for the KISA%.
3. The K value used must exceed $47.2D$ (more than two SD greater than seen in normal controls). For K values less than $47.2D$, the value of 1.0 is substituted in the calculation.

This index was calculated for all 86 keratoconus patients in the Cedars-Sinai database. The eye with the lowest KISA% value that still had minimal clinical signs of keratoconus (ie, scissoring of the red reflex) and a topographic pattern consistent with keratoconus had a KISA% of 30900. Because the goal was to create an index in which a patient with minimal clinical features of keratoconus had a score as close to 100% as possible, this index was di-

vided by 300, giving this patient a KISA% value of 103%. The KISA% index is thus calculated as follows:

$$\text{KISA}\% = (\text{K}) \times (\text{I-S}) \times (\text{AST}) \\ \times (\text{SRAX} \div 300) \times 100$$

20 families with autosomal dominant keratoconus and no other associated genetic disease. All families originated from a specific area in Finland with a high incidence of keratoconus and were thought to have a common founder. A genome-wide screen demonstrated linkage to 16q22.3–q23.1, between markers D16S2624 and D16S3090 with a maximum parametric logarithm of the odds ratio (LOD) score of 4.10 and a nonparametric score of 3.27 ($P=0.00006$). They suggest that this single locus for familial autosomal dominant keratoconus without heterogeneity is the location of the causative gene for keratoconus [51]. Unfortunately there seem to be no genes in this region that could explain the development of keratoconus. Investigators at Cedars-Sinai Medical Center studied this gene region in a single large family with autosomal dominant keratoconus and definitively excluded linkage for a keratoconus gene in this region. This locus therefore seems to be specific for the Finnish families studied, and more work is required to confirm and refine this locus before a gene for keratoconus in this region can be conclusively identified.

Fullerton and coworkers [36] used a novel identity-by-descent approach to study eight individuals from Burnie, a coastal town in northwest Tasmania, Australia, where the incidence of keratoconus is five times that found in the general population. It was assumed that the individuals studied were likely to be related through a founder effect and that only seven or eight generations separated this population from its founders. A genome-wide search of the eight unrelated individuals with keratoconus from this area suggested an association of keratoconus with marker D20S119 at 20q12, where the deviation from Tasmanian allele control frequency distributions ($P = 2.3 \times 10^{-5}$) and a small conserved founder haplotype indicate association. A nearby candidate gene *MMP-9* was excluded as a causative gene, and no other candidate genes in exist in this region that might play a role in the pathogenesis of keratoconus exist in this region.

Rabinowitz et al [47] in the Cedars-Sinai laboratory studied a large family with autosomal dominant kera-

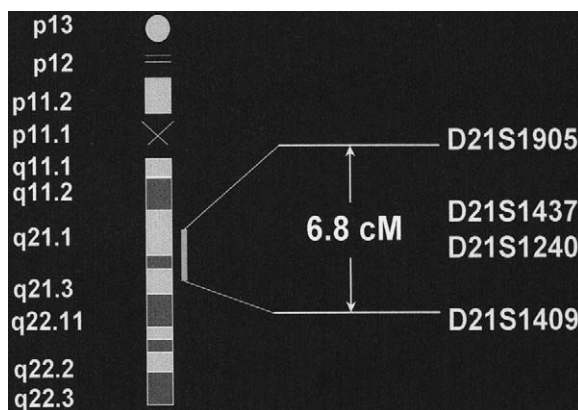


Fig. 6. Location of a putative gene locus for keratoconus on chromosome 21 in one family with autosomal dominant keratoconus.

5-cM intervals is being conducted in this large family and in several other families with autosomal dominant keratoconus. Several other loci with strong linkage have been identified on other chromosomes (none of which have been reported to date), suggesting that multiple gene loci play a role in the pathogenesis of keratoconus.

A recent study by Heon et al [52] suggests that mutations in the *VSX1* homeobox gene (mapped to chromosomal region 20p11–q11) may play a role in up to 4.7% of patients with isolated keratoconus. In one patient with isolated keratoconus requiring a corneal transplant, a *VSX1* mutation (*R166W*) was detected. In a second patient with autosomal dominant keratoconus, a second mutation in the *VSX1* gene (*L159M*) was detected. This mutation segregated with the disease phenotype in three other affected family members and was absent in 277 normal controls, suggesting that it is a disease-causing alteration. Human *VSX1* is a member of the *Vsx1* group of vertebrate paired like-homeodomain transcription factors. The *VSX1* gene was first identified in goldfish, and orthologues have been identified in the zebrafish, chicken, cow, and mouse. In situ hybridization in these species has shown that *vsx1/VSX1* is expressed in the outer tier of the nuclear retina, suggesting that it plays a role in the development of bipolar interneurons [53]. In humans, *VSX1* mRNA has been detected in the inner nuclear layer of the retina, in embryonic craniofacial tissue, and in the adult cornea [54]. In the mouse, *vsx1* expression is first detectable by in situ hybridization at postnatal day 5 and is later restricted to cone bipolar cells in the adult mouse retina. *VSX1* was localized to 20p11–q11 region, and its five exons are distributed

across a 6.2-kb coding sequence. Because of the ocular expression of *VSX1* and its chromosomal localization, Hayashi's group [53] selected it as a candidate gene for posterior polymorphous dystrophy, which has been mapped to the same region. This study was extended to isolated keratoconus because a mutation in this gene was detected in a patient who had both posterior polymorphous dystrophy and keratoconus. These investigators subsequently performed reverse-transcription polymerase chain-reaction studies on RNA isolated from adult cornea. *VISX1* was not detected in the corneal cDNA obtained from two different adult corneas.

In the most extensive gene library of the human adult cornea to date, made by the author's group in collaboration with the National Eye Institutes of Health, the investigators have been unable to detect the pres-

Table 3
Potential loci for keratoconus genes

Locus	References
For isolated keratoconus	
16q22.3-q23	[51]
20q12	[36]
21p	[47]
For keratoconus associated with other disorders	
20p11-q11 (<i>VSX1</i> gene)	[52]
17p13 Leber's congenital amaurosis (<i>LCA4</i> gene)	[56]
17p Leber's congenital amaurosis <i>AiPL 1</i> gene	[57]

ence of the *VISX1* gene in the adult human cornea (Y.S. Rabinowitz, unpublished data). Heon [52] suggests that her inability to detect the *VISX1* gene in the adult cornea suggests that the *VISX1* gene is normally expressed in the developing cornea but not in the adult cornea. Alternatively, she suggests that *VISX1* may be required in noncorneal cell types for the production of a signaling molecule that is essential for normal corneal development or maintenance. A possibility that she does not discuss is that her patients had subtle retinal disease that would put this disease outside the realm of isolated keratoconus. In her report, patients with posterior polymorphous dystrophy had abnormal electroretinogram results, but there is no evidence that electroretinograms were performed on the patients with isolated keratoconus studied by her group. Keratoconus is strongly associated with retinal disease, in particular Leber's congenital amaurosis [55]. Gene loci for families with keratoconus and Leber's congenital amaurosis have been identified on chromosome 17 (Table 3), but there is no evidence from these studies that any genes in these regions play a role in isolated keratoconus [56,57].

The role of eye rubbing

Eye rubbing occurs more frequently in patients with keratoconus (84%) than in the general population (52%) [13]. Formal genetic studies using segregation analysis have demonstrated that eye rubbing by itself cannot cause keratoconus, because eye rubbing is an environmental factor, and keratoconus has now clearly been shown to be a genetic disease [13,32]. This association does, however, raise the question as to whether keratoconus patients have a genetic predisposition to eye rubbing or possibly a genetic keratocyte hypersensitivity to the trauma of eye rubbing. The reasons for the association between eye rubbing and the pathogenesis of keratoconus is unknown. It has been suggested that chronic epithelial damage can induce chronic keratocyte apoptosis, which has been shown to be much higher in the keratoconic cornea. Kim et al [58] detected apoptotic keratocytes in 60% of keratoconic corneas, compared with 35% of corneas with stromal dystrophies and 0% in normal controls. Rubbing the eye is a behavior that might chronically activate apoptosis through inflammatory mediators and lead to the onset and progression of keratoconus [59]. As the identification of genes that contribute to keratoconus improves through better means of identifying early phenotypes and with the aid of new molecular genetic technology, the contribution of eye rubbing and its role, if any, in the pathogenesis of keratoconus

will become more evident. Given the complex nature of this disorder that clearly is not a single-gene defect, eye rubbing might turn out to be an environmental trigger, acting in concert with multiple complex gene interactions, that contributes to the development of keratoconus. The evidence, currently, however is purely circumstantial.

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