Genetic Epidemiological Study of Keratoconus:
Evidence for Major Gene Determination

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Keratoconus (KC) is a noninflammatory corneal thinning disorder and the major cause of cornea transplantation in the Western world. Genetic factors have been suggested in the cause of KC. We conducted a family study to investigate genetic contributions to the development of KC by evaluating familial aggregation and testing genetic models with segregation analysis. KC was diagnosed based on clinical criteria. Familial aggregation of KC was evaluated using both clinical status and three videokeratography indices generated by the Topographic Modeling System (TMS-1). The estimated KC prevalence in first-degree relatives was 3.34% (41/1,226, 95% CI: 3.22–3.46%), which is 15 to 67 times higher than that in the general population (0.23–0.05%). For all three videokeratography indices, CK, IS, and KISA, KC propositi had significantly higher mean values than controls (all \( P < 0.0001 \)). Clinically unaffected parents also had significantly higher values for these indices than controls (all \( P < 0.016 \)). The correlation of KISA in sib and parent-offspring pairs (\( r = 0.30 \) and 0.22, respectively, both \( P < 0.0005 \)) was significantly greater than that in marital pairs (\( r = 0.14 \)), and the latter was not significantly different from zero. We performed segregation analysis on KISA in 95 families ascertained through KC propositi. Hypotheses of both sporadic and environmental models were rejected (\( P < 0.001 \)); a major gene model was not rejected (\( P > 0.1 \)). Additionally, the most parsimonious model was autosomal recessive. In conclusion, we observed strong evidence of familial aggregation in KC and its subclinical indices and this aggregation is likely due to a major gene effect. Am. J. Med. Genet. 93:403–409, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: keratoconus; videokeratography indices; familial aggregation; segregation analysis; major gene

INTRODUCTION
Keratoconus (KC) is a noninflammatory progressive thinning disorder of the cornea, which leads to progressive mixed myopic and irregular astigmatism. The estimated prevalence of KC is approximately 50–230/100,000 in the general population [Rabinowitz, 1998]. KC occurs in all ethnic groups with no significant gender difference. The age of onset is at puberty, and the disorder is progressive until the third to fourth decade of life when it usually arrests. It is a major cause of cornea transplantation in developed countries. The underlying biochemical processes and its cause remain poorly understood. By far the most common presentation is non-syndromal KC. However, the association between KC and some rare genetic disorders has been reported. For example, Gullen and Butler [1963] recognized that Down syndrome was associated with KC. Elder [1994] reported Leber congenital amaurosis and its association with KC. KC is also associated with atopy, eye rubbing, and hard contact lens wearing [Gasset et al., 1978; Grayson and Keates, 1969; Karseras and Ruben, 1976; Macsai et al., 1990].

Although the cause of KC is unknown, there are sev-
eral lines of evidence suggesting a genetic component. These include its occurrence in relatives in second and third generations [François, 1961], a positive family history in 6 to 10% of KC cases [Rabinowitz, 1998], and its higher concordance rate in monozygotic twins (six of eight sets of identical twins had clinical keratoconus observed in both twins) [Parker et al., 1996].

Computer-assisted videokeratography has been used to develop quantitative indices to characterize KC and to identify subclinical phenotypes [Rabinowitz, 1995; Rabinowitz and McDonnell, 1989]. This device uses 25 illuminated rings to span most of the surface of the cornea from the apex to the limbus and generates videokeratographs for each eye. Videokeratographs are then analyzed with a computer software that automatically calculates three quantitative indices: central K (CK), describing the central corneal steepness; the I-S value (IS), measuring inferior-superior dioptic power asymmetry; and KISA, combining CK, IS, and two other measures that quantify the irregular shape and astigmatism of the cornea. It has been shown that keratoconus is more accurately distinguished from the normal condition by videokeratography-derived indices than by other measurements [Rabinowitz et al., 1998]. These quantitative videokeratography indices might serve as subclinical phenotypes, which could occur in the process of the development of KC. Using subclinical phenotypes can greatly aid genetic studies. First, because there might be reduced penetrance or variability of phenotype expression, the use of subclinical phenotypes may allow us to detect the abnormal genotype in the absence of clinical disease. Second, subclinical phenotypes may also help to distinguish between an inherited predisposition and a secondary abnormality due to the disease process. If we find that subclinical abnormalities in unaffected relatives are similar to those found in the propositi, it suggests that such a phenomenon is an inherited predisposition, not a secondary abnormality due to the disease process.

In this study we used both disease-affection status and subclinical phenotypes, i.e., videokeratography indices, to study the familiality of KC and the role of genetic factors in KC. We performed analyses in three phases: 1) to test if there is familial aggregation in KC; 2) to test whether a major gene contributes to this aggregation; and 3) to examine whether there is evidence for any specific pattern of inheritance in KC.

**MATERIALS AND METHODS**

The KC propositi, index cases through whom the families were recruited, were sequentially ascertained at the Cornea Genetic Eye Institute at Cedars-Sinai Medical Center from 1992 to 1996. The first-degree relatives of propositi were recruited for the study. Normal controls, with no known clinical evidence or family history of KC, were recruited from spouses or acquaintances of KC patients, as well as employees of Cedars-Sinai Medical Center.

The diagnosis of KC was based on clinical examination. Any patient who had one or more of the following clinical signs with no other pathology in one eye was classified as KC: obvious corneal stromal thinning, Vogt striae, or a Fleischer ring detected by slit-lamp examination; obvious scissoring of the red reflex or the Charleaux oil droplet sign was identified by retinoscopy.

Propositi with a family history of Down syndrome, Leber congenital amaurosis, or any other recognized genetic disorder were excluded from this study in order to disentangle the contribution of heredity to the development of isolated KC. Eyes with complicating factors, such as cornea transplantation, scarring, or contact lenses wearers, were also excluded from the analysis to avoid the videokeratography artifacts. Moreover, individuals age 13 years or younger were not included in the analysis because they may not be old enough to develop KC.

In total we ascertained 539 propositi, 1,226 first-degree relatives, and 268 controls. We interviewed each individual with a questionnaire including information on demography, medical history, and family history of KC. Of these subjects, 381 KC propositi, 373 of their first-degree relatives, and 252 controls underwent both clinical and videokeratography evaluation. The clinical examinations included slit-lamp biomicroscopy, retinoscopy examinations, and fundus evaluation. The slit-lamp biomicroscope was used to examine whether there was stromal corneal thinning, Vogt striae, or a Fleischer ring. Retinoscopy was performed with a fully dilated pupil (20 minutes after phenylephrine 2.5% and cyclopentolate 1% drops had been installed in the eye) to determine the presence or absence of retroillumination signs of keratoconus, such as the oil droplet sign and the scissoring. Videokeratography evaluation was also performed on each eye. The study protocol was approved by the Institutional Review Board of Cedars-Sinai Medical Center.

**Videokeratography Measurements**

All eyes were studied using the Topographic Modeling System (TMS-1) (Computed Anatomy, Inc., New York, NY), a computerized corneal topographic analysis system (Software Version 1.61). Indices of CK, IS, and KISA on each eye were generated based on this system, which has been described in detail [Rabinowitz, 1995; Rabinowitz and Rasheed, 1999]. CK, a measure of central corneal steepness, is calculated by averaging the dioptic power points on rings 2, 3, and 4 of the videokeratograph. In total 768 data points were evaluated. The IS value, a measure of corneal asymmetry, is calculated by subtracting the superior (S) value from the inferior (I) value. The I value is calculated by averaging 15 data points on rings 14, 15, and 16 of the videokeratographs approximately 3.0 mm inferior to the center of the cornea at 30 degree intervals (i.e., at axes 210, 240, 270, 300, and 330 degree); the S value is derived from 15 data points at 30, 60, 90, 120, and 150 degrees on the superior cornea. A positive IS value indicates a steeper inferior cornea, and a negative IS value indicates a steeper superior cornea. The KISA index is derived from the product of four indices that include CK, IS, and two other measurements (AST and SRAX) that quantify the irregular shape and astigmatism of the cornea, i.e., KISA = (CK) × (I − S) ×
paring these quantitative indices between two groups. Software were used [SAS Institute Inc., 1990] for the computation of means of KISA associated with each underlying type of individuals, namely AA, AB, and BB. The types (denoted A and B) were assumed as three possible environmental components. Two alleles at a single locus of the major gene model" consists of a major gene effect, polygenes, or environmental factors. A "general model" assumes no familial correlation, i.e., parameters \( \rho_{sp} \) and \( \rho_{po} \) denoted spouse-spouse and parent-offspring correlation, respectively. The "sporadic" model assumed no familial correlation, i.e., parameters \( \rho_{sp} \) and \( \rho_{po} \) were fixed to be 0. The "environmental" model restricted family correlation, i.e., parameters \( \rho_{sp} \) and \( \rho_{po} \) were free and were estimated. Hypotheses were tested by comparing the general model with various restricted models. Two criteria were used to compare these models. For hierarchical models, the likelihood ratio test (LRT) was used. Twice the difference in log-likelihoods \((-2\ln(L))\) between a restricted and an unrestricted model can be treated as a chi-square statistic with degrees of freedom equal to the difference in the number of parameters being estimated under the two competing models. For non-hierarchical models, Akaike’s Information Criterion (AIC) was used [Akaike, 1974]. AIC = \(-2\ln(L) + 2k\), where k is the number of parameters estimated in the models. Because this statistic combines measures of parsimony and goodness of fit, the hypothesis with the minimum AIC best fits the data.

Since families were recruited through the propositi, ascertainment correction was necessary in order to estimate the meaningful parameters for the population. We corrected for ascertainment by conditioning on the propositi in the analysis.

RESULTS
Study Population

The study included individuals between age 13 and 97 years. Table I shows the age and gender distributions in propositi, their first-degree relatives, and controls. The mean age for propositi, their first-degree relatives, and controls were 36.3, 45.4, and 39.4 years, respectively. More males were observed in propositi (60.1%) than in controls (46.2%) in this study population.

TABLE I. Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Propositi</th>
<th>First-degree relatives</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>539</td>
<td>1,226</td>
<td>268</td>
</tr>
<tr>
<td>Age (years) Mean (SD)</td>
<td>36.3 (13.0)</td>
<td>45.4 (18.6)</td>
<td>39.4 (12.5)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>60.1</td>
<td>49.7</td>
<td>46.2</td>
</tr>
</tbody>
</table>
tion. The number of males and females was approximately the same among first-degree relatives (49.7% were males).

**Quantitative Measurements**

Indices CK, IS, and KISA were normally distributed in controls. The KISA index exhibited a bimodal distribution in the whole sample, including propositi, relatives, and controls. The means of the two peaks were 3.5 D and 7.9 D (Fig. 1a). Indices CK and IS also showed similar distributions, but the bimodality was not as obvious as with KISA (Fig. 1b and c). The bimodal distribution of these traits is consistent with the major gene involvement hypothesis, especially for the KISA index.

The means of three quantitative indices in KC propositi and normal controls are compared in Table II. The average values of CK, IS, and KISA were significantly elevated in propositi (51.56 D, 7.51 D, and 7.88 D) compared with controls (43.95 D, 0.41 D, and 2.92 D, respectively) (all $P < 0.0001$). These data, together with previously reported discriminant analysis in a subset of the current sample [Rabinowitz et al., 1998], indicate that the quantitative videokeratography indices can be used to distinguish KC from normal individuals.

**Familial Aggregation**

The estimated KC prevalence in first-degree relatives was 3.34% (41/1,226, 95% CI = 2.33–4.35%). This is 15 to 67 times higher than the general population prevalence (0.23–0.05%) [Rabinowitz, 1998]. Among first-degree relatives, the empiric risk of KC was 3.78% (23/609, 95% CI = 2.28–5.29%) in sibs, and 2.92% (18/617, 95% CI = 1.58–4.22%) in parents and offspring. The increased empiric risks in relatives of KC patients suggest a strong familial aggregation of KC affection status.

For quantitative indices of KC, we compared clinically unaffected relatives with controls. Table III summarizes the means and standard deviations of the three indices for the clinically unaffected relatives, unaffected parents only, and controls. A consistent trend was observed in that clinically unaffected relatives had higher mean values than controls for all three indices. Clinically unaffected parents had significantly increased mean values as compared with controls for all three indices (all $P < 0.016$ or less). Such results provide further support not only for the familial aggrega-

![Fig. 1. Distributions of three quantitative indices in all subjects.](image-url)
tion of KC, but also for KC related quantitative traits. Because the relatives of KC patients have a greater risk to develop KC than the general population, the increased quantitative indices in relatives provide evidence that these indices may serve as subclinical phenotypes of KC.

Table IV shows the familial correlation of the three indices among all unaffected relatives. The correlations of KISA between sib and parent-offspring pairs (r = 0.30 and 0.22, respectively) are significantly greater than that in marital pairs (r = 0.14) (the latter is not significantly different from zero). The estimated upper-bound heritability for KISA was 0.6. These results suggest that the familial aggregation in KC is more likely due to genetic factors.

Segregation Analysis

In total, 95 families with at least three individuals with the KISA measurement were used in the segregation analysis. The estimated parameters, −2Ln(L), χ², and the AIC statistics for each model are summarized in Table V. First, we compared the sporadic model (model 2), the environmental model (model 3), and the Mendelian model (model 7) to the general model (model 1). The Mendelian model was not rejected compared with the general model (model 7 versus model 1, P > 0.1). In contrast, both the sporadic and environmental models were significantly different from the general model (models 2 and 3 versus model 1) and were rejected at P < 0.001. Second, we compared data within the major gene models. The major gene-only model was not significantly different from the major gene-plus-environmental model, the major gene-plus-polygene model, and the major gene with polygene and environmental model (model 7 versus models 4, 5, and 6, all P > 0.05, P-values not shown in the table). This suggested that a major gene could play a prominent role in the transmission of KISA. Finally, we further tested the mode of inheritance under the assumption of Mendelian segregation, the result indicated that the recessive model was the best fitting model, i.e., it was not significantly different from the major gene model (model 9 versus model 7, P > 0.1). In contrast, both the dominant and additive models were significantly different from the major gene model (models 8 and 10 versus model 7, both P < 0.01). The most parsimonious model was the Mendelian recessive model (AIC = 1,006.381, the smallest value). The estimated frequency of allele A was 0.37, and the estimated KISA means were 3.93, 2.83, and 2.83 D for AA, AB, and BB genotypes, respectively. Thus, the complex segregation analysis demonstrated that the autosomal-recessive model fit the data best among the tested models.

DISCUSSION

We have demonstrated strong evidence for familial aggregation of KC and its quantitative measures. Segregation analysis of KISA index showed that a major gene model best fits these data. Autosomal-recessive inheritance for the KISA index was suggested. Thus, our study has provided some insights into the understanding of familiality in KC.

This study consistently showed that the videokeratography indices, CK, IS, and KISA, were good quantitative measures for KC affection [Rabinowitz et al., 1998]. Both KC and its quantitative measurements showed familial aggregation in this study population. This is consistent with results of a previous study [Tretter et al., 1995] in which family history was identified as one of the risk factors for KC. The estimated KC prevalence in first-degree relatives was 3.34%, which is much higher than the prevalence in the general population (0.05–0.23%). The significantly increased correlations of KISA in sib and parent-offspring pairs as compared with marital pairs not only support the familial aggregation of KC but also suggest the importance of genetic factors.

To our knowledge, the present study is the first to apply complex segregation analysis to KC pedigree

### Table III. Comparison of KC Indices Between Relatives and Controls

<table>
<thead>
<tr>
<th>Indices</th>
<th>N</th>
<th>Mean (SD)</th>
<th>N</th>
<th>Mean (SD)</th>
<th>N</th>
<th>Mean (SD)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK (D)</td>
<td>346</td>
<td>44.73 (1.63)</td>
<td>142</td>
<td>44.70 (1.69)</td>
<td>252</td>
<td>43.95 (1.44)</td>
<td>0.0001</td>
</tr>
<tr>
<td>IS (D)</td>
<td>346</td>
<td>0.74 (0.98)</td>
<td>142</td>
<td>0.89 (1.27)</td>
<td>251</td>
<td>0.41 (0.50)</td>
<td>0.0001</td>
</tr>
<tr>
<td>KISA (D)</td>
<td>328</td>
<td>2.93 (1.10)</td>
<td>134</td>
<td>3.20 (1.14)</td>
<td>247</td>
<td>2.92 (0.96)</td>
<td>0.016</td>
</tr>
</tbody>
</table>

*Parents are included.

P value is based on a comparison of unaffected parents versus controls.

### Table IV. Familial Correlation of KC Indices in Unaffected Individuals

<table>
<thead>
<tr>
<th>KC Indices</th>
<th>N</th>
<th>r</th>
<th>P value*</th>
<th>N</th>
<th>r</th>
<th>P value*</th>
<th>N</th>
<th>r</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS (D)</td>
<td>49</td>
<td>-0.12</td>
<td>ns**</td>
<td>249</td>
<td>0.16</td>
<td>0.01</td>
<td>187</td>
<td>0.24</td>
<td>0.0009</td>
</tr>
<tr>
<td>CK (D)</td>
<td>49</td>
<td>0.09</td>
<td>ns</td>
<td>249</td>
<td>0.06</td>
<td>ns</td>
<td>187</td>
<td>0.15</td>
<td>0.04</td>
</tr>
<tr>
<td>KISA (D)</td>
<td>49</td>
<td>0.14</td>
<td>ns</td>
<td>249</td>
<td>0.22</td>
<td>0.0005</td>
<td>187</td>
<td>0.30</td>
<td>0.00003</td>
</tr>
</tbody>
</table>

*Correlation coefficient.

P value was based on comparison of each r to r = 0.

**Statistically not significant.
data to investigate the existence of a major gene effect and the mode of inheritance of a KC related index. Segregation analysis of KISA suggested Mendelian inheritance, i.e., the major gene model was not rejected from the most general model. An autosomal recessive transmission model fits our data best. In contrast, hypotheses of sporadic and environmental models were strongly rejected in this study. Rejection of the sporadic model was also consistent with the findings of familial aggregation demonstrated by increased empiric risks in relatives and increased correlation of quantitative indices in relative pairs.

The bimodal distribution of KC subclinical indices, the familial correlation of both clinical and subclinical measures of KC, and the segregation analysis of KISA all together support the hypothesis of major gene involvement in the genetic mechanisms of KC.

Although the recessive model is suggested by these data, there may be genetic heterogeneity as well. For example, we have four large pedigrees referred from other clinics (not included in this study) with a clearly dominant inheritance pattern. Furthermore, segregation analysis does not resolve whether one or more loci are involved. These considerations suggest that non-parametric or model-free methods will likely be the most useful approach in linkage analysis to identify disease susceptibility gene or genes.

The data presented in this study showed strong evidence for familial aggregation of KC with respect to affection status and KC related quantitative indices. Segregation analysis provided evidence that this aggregation was well explained by a major gene effect with recessive transmission. Our study also suggests that such indices might have potential as predictors for the early detection of KC in a quantifiable and reproducible manner. Moreover, these quantitative indices may be used as subclinical phenotypes for KC to facilitate the gene mapping endeavors for this disorder. Our findings warrant further investigation of the genetic mechanisms in KC and provide a foundation for future gene mapping studies.

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