Variation in the Lysyl Oxidase (LOX) Gene Is Associated with Keratoconus in Family-Based and Case-Control Studies

Yelena Bykhovskaya,¹ Xiaohui Li,² Irina Epifantseva,¹ Talin Haritunians,² David Siscovick,³ Anthony Aldave,⁴ Loretta Szczotka-Flynn,⁵ Sudha K. Iyengar,⁵ Kent D. Taylor,² Jerome I. Rotter,² and Yaron S. Rabinowitz^{1,2,6}

PURPOSE. Keratoconus is a bilateral noninflammatory progressive corneal disorder with complex genetic inheritance and a common cause for cornea transplantation in young adults. A genomewide linkage scan in keratoconus families identified a locus at 5q23.2, overlapping the gene coding for the lysyl oxidase (LOX). LOX encodes an enzyme responsible for collagen cross-linking in a variety of tissues including the cornea. Corneal collagen cross-linking with long-wave ultraviolet light and riboflavin is a promising new treatment for keratoconus. To determine whether LOX is a genetic determinant of the pathogenesis of keratoconus, we analyzed association results of LOX polymorphisms in two independent case-control samples and in keratoconus families.

METHODS. Association results were analyzed of single-nucleotide polymorphisms (SNPs) in the LOX gene from a Genome-Wide Association Study (GWAS) investigation in two independent panels of patients with keratoconus and controls and in keratoconus families.

RESULTS. Evidence of association was found at SNPs rs10519694 and rs2956540 located in intron 4 of LOX in the GWAS discovery case-control panel with P values of 2.3×10^{-3} and 7×10^{-3} , respectively. The same two SNPs were found to be associated with keratoconus by family-based association testing with P values of 2.7×10^{-3} and 7.7×10^{-4} , respectively. Meta P values of 4.0×10^{-5} and 4.0×10^{-7} were calculated for SNPs rs10519694 and rs2956540 by analyzing case-control and family samples simultaneously. Sequencing of LOX exons in a subset of keratoconus patients identified two polymorphisms, rs1800449 and rs2288393, located in LOX transcripts I and II, associated with keratoconus in case-control and family samples with a meta P value of 0.02.

CONCLUSIONS. Results provided strong genetic evidence that LOX variants lead to increased susceptibility to developing of keratoconus. (*Invest Ophthalmol Vis Sci.* 2012;53:4152–4157) DOI:10.1167/iovs.11-9268

From the ¹Regenerative Medicine Institute, Ophthalmology Research, Department of Surgery, Division of Surgical Research, Cedars-Sinai Medical Center, Los Angeles, California; ²Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, California; ³Departments of Medicine and Epidemiology and Cardiovascular Health Research Unit, University of Washington, Seattle, Washington; ⁴The Jules Stein Eye Institute at University of California Los Angeles, Los Angeles, California; ⁵University Hospitals Eye Institute, Department of Ophthalmology and Visual Sciences, Case Western Reserve University, Cleveland, Ohio; and the ⁶Cornea Genetic Eye Institute, Cedars-Sinai Medical Center, Beverly Hills, California.

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Corresponding author: Yaron S. Rabinowitz, Regenerative Medicine Institute, Ophthalmology Research, Department of Surgery, Division of Surgical Research, Cedars-Sinai Medical Center, 8700 Beverly Blvd, Steven Spielberg Building Suite 339, Los Angeles, CA 90048; yaron.rabinowitz@cshs.org.

Keratoconus is a pathologic condition in which the cornea assumes a conical shape as a result of noninflammatory thinning and protrusion. It is also a common cause of corneal transplantation in young adults. Occasionally, keratoconus is associated with other rare genetic disorders; however, isolated keratoconus is by far the most common presentation seen by the practicing clinician.¹ Although keratoconus cases have frequently been reported as sporadic in the past, twin studies, reports of familial aggregation, and formal segregation analysis² provide indisputable evidence that genetic factors play a crucial role in the pathogenesis of isolated keratoconus.³

To identify genomic locations of susceptibility genes for keratoconus we conducted a two-stage genomewide linkage study using the nonparametric method.⁴ We observed evidence of linkage for keratoconus on chromosomes 4, 5, 9, 12, and 14. After looking at biological functions of hundreds of known or predicted genes in the linkage regions, we found that only a few of them constituted plausible keratoconus candidate genes. The most promising one is a gene encoding the enzyme lysyl oxidase (LOX) located under a linkage peak at 5q23.2. Lysyl oxidase initiates the cross-linking of collagens and elastin by catalyzing oxidative deamination of the epsilon-amino group in certain lysine and hydroxylysine residues.⁵ A promising new treatment called corneal collagen cross-linking (CXL), which uses a combination of riboflavin and long-wave ultraviolet light to increase the collagen cross-links in the cornea, thereby stiffening

TABLE 1. Oligonucleotide Primers Used for PCR Amplification and Sequencing of LOX Gene Exons

	Forward Primer	Reverse Primer	Length of the product (bp)	Annealing Temperature (°C)
Exon 1-1	CTTAACGCTCCCTGTGCAACG	GCGCTGTCTGGTTCTCCG	901*	61.2
Exon 1-2	CCGTCACCTGGTTCCAAGCTG	ACGTCGAGAAGCCACATAGC	336	60.2
Exon 2	CTGTCTCCGCTCCACTC	ACTTCCCAGCTCTTGTC	363	60.8
Exon 2 alt	CCAGCTATGTGGCTTCTCGAC	ACTTCCCAGCTCTTGTC	543	61.6
Exon 3	TTGGGAAAGGAGGATTGTCACTAC	TCCCTTCAGGTAAGAAATAAGACT	343	52.7
Exon 4	ATTTGGTCTCAATTTTAATGTG	ATGCTATTTAATGCTAACTAACGG	358	53.1
Exon 5	AAATACTTCTCAAGGGAGATGA	GTATAATTGCTTCCAATACCATG	352	49.9
Exon 6	AACGTCTCCAGAGTTTAACCA	GCATACCATTTTCTGCCTTTG	388	53.2

* PCR product exon1-1 was also sequenced with internal primers TCCGCTCGCTCCTTGTTG and TCGGGCCTTGCAGCTCT.

it, to halt the progression of keratoconus, is currently being used with promising results.⁶⁻⁸

In this study we evaluated polymorphisms in the *LOX* gene by genetic association testing in two independently collected case-control panels of individuals with keratoconus and in families with keratoconus. We found evidence of association at two single-nucleotide polymorphisms (SNPs), rs10519694 and rs2956540, located in intron 4 of *LOX* in the Genomewide Association Study (GWAS) discovery case-control panel, with *P* values of 2.3×10^{-3} and 7×10^{-3} , respectively, which were further confirmed in confirmation case-control and family-based analysis with meta *P* values of 4.0×10^{-5} and 7.7×10^{-4} , respectively, thus providing strong genetic evidence that *LOX* variants lead to increased susceptibility for developing of keratoconus.

MATERIALS AND METHODS

Subjects

Clinically affected patients with keratoconus and their family members were recruited as a part of the longitudinal videokeratography and genetic study² at the Cornea Genetic Eye Institute, Cedars-Sinai Medical Center (Los Angeles, CA). Additional patients were recruited at The Jules Stein Eye Institute (University of California, Los Angeles, CA) and University Hospitals Eye Institute at Case Western Reserve University (Cleveland, OH). Patients diagnosed with forme fruste keratoconus were not included in this study specifically, to avoid any potential confounding results. Institutional Review Board (IRB) approval was obtained at all clinic sites. Written informed consent was obtained from all subjects. The study was conducted in accordance with the provisions of the Declaration of Helsinki.

GWAS: Discovery Case-Control Cohort. Clinically affected Caucasian keratoconus cases (*N* = 240) were enrolled into the GWAS at the Cornea Genetic Eye Institute. After removing samples with poor genotyping quality, 222 samples were included in the analysis. In all, 3324 Caucasian controls were obtained from the Cardiovascular Health Study (CHS), a population-based cohort study of risk factors for cardiovascular disease and stroke in adults 65 years of age or older, recruited at four field centers. A total of 5201 predominantly Caucasian individuals were recruited from 1989 to 1990 from random samples of Medicare eligibility lists, followed by an additional 687 African-Americans recruited from 1992 to 1993 (total *N* = 5888). CHS was approved by the IRB at each recruitment site, and subjects provided informed consent for the use of their genetic information. African-American CHS participants were excluded from analysis due to an insufficient number of ethnically matched keratoconus cases.^{9,10} Individuals recruited for the cardiovascular health study filled out an extensive ophthalmologic questionnaire and were administered a retinal exam by a trained ophthalmologist. With an estimated prevalence between 1:2000¹¹ and 1:1163¹² in the Caucasian population, we expected no considerable keratoconus contribution to the controls' sample.

GWAS: Confirmation Case-Control Cohort. An independent group of 304 independent keratoconus cases and controls was

recruited through a collaborative effort. In all, 232 keratoconus cases were recruited at the Cornea Genetic Eye Institute; 26 cases at the Jules Stein Eye Institute; and 46 cases at University Hospitals Eye Institute; additionally, 518 normal controls were also recruited at the Cornea Genetic Eye Institute.

Keratoconus Families. Family members of keratoconus cases diagnosed at the Cornea Genetic Eye Institute were recruited to perform family-based studies. A total of 307 individuals from 70 families, consisting of 146 keratoconus patients and 161 unaffected family members, were obtained for this study. A total of 186 individuals in 41 pedigrees were Caucasians, whereas 91 individuals in 20 pedigrees were identified as Hispanics.

Combined Panel for Genotyping of Individual SNPs (TaqMan Genotyping). A combined panel of 919 familial and case-control subjects as well as controls was assembled to perform genotyping of individual SNPs. This panel consisted of 377 independent keratoconus cases from GWAS Discovery and Confirmation Cohorts, 114 controls from GWAS Confirmation Cohort, and 428 affected and unaffected individuals from families with keratoconus.

Clinical Diagnosis

The diagnosis of keratoconus was performed by a cornea fellowship trained ophthalmologist based on clinical examination and videokeratography pattern analysis. Clinical examination included slit-lamp biomicroscopy, cycloplegic retinoscopy, and fundus evaluations. Slit-lamp biomicroscopy was used to identify stromal corneal thinning, Vogts' striae, or a Fleischer ring. Retinoscopic examination was performed with a fully dilated pupil to determine the presence or absence of retro illumination signs of keratoconus, such as the oil droplet sign and scissoring of the red reflex 20 minutes after phenylephrine 2.5% and cyclopentolate 1% drops had been instilled in the eye. Videokeratography evaluation was performed on each eye using the topographic modeling system (Tomey TMS-4; Computed Anatomy, New York, NY). Patients were considered as having keratoconus if they had at least one clinical sign of keratoconus and a confirmatory videokeratography map with an asymmetric bowtie with skewed radial axis above and below the horizontal meridian (AB/SRAX) pattern.¹³

Cell Lines and DNA Isolation

Lymphoblastoid cell lines were established from peripheral blood lymphocytes on all study participants and immortalized with Epstein-Barr virus.¹⁴ Genomic DNA was extracted using a tissue core kit (NucleoSpin Tissue kit; Macherey-Nagel Inc., Bethlehem, PA) according to the manufacturer's protocol.

Genotyping

GWAS: Discovery. SNP rs10519694 in the *LOX* gene was genotyped as a part of a whole genome genotyping procedure (HumanCNV370-Quad BeadChip; Illumina Inc., San Diego, CA),^{15,16} following the manufacturer's protocol. In all, 290K SNPs including

TABLE 2. Results of Association Testing of LOX SNPs rs10519694 and rs2956540 in Case-Control Cohorts and Families with Keratoconus

SNP	Position	Discovery Case/Control Panel			Confirmation Case/Control Panel			Families			Meta	
		FA	FU	OR	P	FA	FU	OR	FU	OR	P	P
rs10519694	121,435,118	0.18	0.26	0.67	$\underline{2.3 \times 10^{-3}}$	0.20	0.22	0.86	0.29	0.64	$\underline{2.7 \times 10^{-3}}$	$\underline{4.0 \times 10^{-5}}$
rs2956540	121,437,079	0.36	0.44	0.73	$\underline{7 \times 10^{-3}}$	0.36	0.44	0.70	0.54	0.64	$\underline{7.7 \times 10^{-4}}$	$\underline{2.5 \times 10^{-7}}$

Significant *P* values are underlined.

rs10519694 passed rigorous quality control procedures. They included genotyping rate per subject > 99%, narrow distribution (theta SD), and wide separation between genotyping clusters (R/theta plots), minor allele frequency > 5%, Hardy-Weinberg test (SNPs with *P* value < 0.001 were excluded). SNPs were also excluded from analysis for significant differences in missing data between cases and controls (SNPs with *P* value in missingness test < 0.01 were excluded).

GWAS: Confirmation. Five SNPs in the *LOX* gene were genotyped in the confirmation cohort using commercial software (iSelect Infinium BeadChip¹⁷; Illumina Inc.), containing 4905 SNPs, of which 4650 SNPs remained following clustering quality control. The average genotyping rate for samples genotyped (on the iSelect platform) and passing quality control was 99.98%. Genotyping concordance among 20 replicated samples was 100%.

TaqMan Genotyping. SNPs rs1800449 and rs2956540 were genotyped using SNP-specific predesigned TaqMan genotyping assay (Applied Biosystems Inc., Carlsbad, CA). Allelic discrimination was performed on a sequence detection system (ABI 7900; Applied Biosystems).

Imputation of Genotyping Data in CHS Controls Panel. An open-source genotype imputation software (IMPUTE version 2.1.0) was used to perform imputation of the genotyping data of SNP rs2956540 in CHS Caucasian controls (using HapMap Phase I and II data, release #22, National Center for Biotechnology Information [NCBI] Build 36, as the reference panel).¹⁸

Statistical Analyses

Association Testing of GWAS Discovery and Confirmation Case-Control Data. Odds ratios (ORs) and their standard errors (SEs) were calculated using genomewide SNP data under logistic regression models using a free, open-source whole genome association analysis toolset (PLINK program, v1.07; <http://pngu.mgh.harvard.edu/purcell/plink/>).¹⁹ Principal component analysis for population stratification was tested using principal component analysis software (EIGENSTRAT computer program).²⁰ Sex and principal components variables were used as covariates. Because of the large age difference between patients and controls in the discovery panel, age was not used as a covariate.

Association Testing of Family Data. Association testing in families was done using generalized estimation equation model accounting for pedigree correlations implemented in Genome-Wide Association analyses with Family package²¹ (GWAF).

Meta-Analysis. Meta-analysis of associated SNPs in discovery, confirmation, and family samples was calculated using inverse-variance weighting (PLINK).

Sequencing

From 16 patients with keratoconus, of which 13 were familial cases and 3 were individual cases from discovery case-control panel, we selected to perform *LOX* gene sequencing. A complete coding region of *LOX* gene, including 5' UTR, 3' UTR, and splice sites (intron-exon junctions), was amplified by PCR and sequenced using forward and reverse primers as shown in Table 1. Primers were designed using primer analysis software (OLIGO 7 program; Molecular Biology Insights, Cascade, CO). Polymerase chain reaction (PCR) amplifications were performed in a PCR system (GeneAmp PCR System 9700; Applied Biosystems) using optimal annealing temperatures (calculated by OLIGO). Amplified DNA was separated by gel electrophoresis and extracted using a commercial kit (QIAquick Gel Extraction kits; Qiagen, Valencia, CA). Extracted DNA was sequenced using a commercial kit (Big Dye Terminator Ready Reaction kits; Applied Biosystems) on a capillary analyzer (3730 DNA Analyzer; Applied Biosystems). Sequencing results were visualized using Sequence Scanner V1.0 and aligned to the genomic sequence using the NCBI BLAST 2 (Basic Local Alignment Search Tool) program.

TABLE 3. Patients with Keratoconus Used for *LOX* Gene Sequencing

#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Status	Fam	Fam	Fam	Fam	Fam	Ind	Fam	Ind	Ind	Fam	Fam	Fam	Fam	Fam	Fam	Fam
Sex	F	M	M	F	F	F	M	F	F	M	F	F	F	M	F	F

Individuals heterozygous for rs1800449 (rs2288393) are marked in bold. Ind, individual case from discovery case-control panel; Fam, familial case.

RESULTS

Genetic Association Testing of *LOX* Polymorphisms in Case-Control GWAS Study

To test whether a variation in the *LOX* gene is associated with keratoconus, we examined the results of a comprehensive genomewide case-control study performed in two large independent panels of case-control subjects.²² We found that SNP rs10519694 located in the intron 4 of *LOX* was associated with keratoconus in the discovery cohort of the GWAS study with a suggestive *P* value of 2.3×10^{-3} (Table 2). To test for variations in the *LOX* gene in a replication cohort, we genotyped SNPs rs10519694, rs2956540, rs3792803, rs34226665, and rs2434980, all located in the *LOX* gene (using the custom iSelect Infinium BeadChip). We found the same direction of the effect at rs10519694 in the case/control replication panel; however, the difference in allele frequencies was not statistically significant (Table 2). Interestingly, a positive association signal was observed in the replication panel for SNP rs2956540, also situated in the intron 4 of *LOX*, with a *P* value of 3.5×10^{-3} (Table 2). The distance between rs10519694 and rs2956540 is approximately 2 KB; these SNPs are in linkage disequilibrium, with $r^2 = 0.6$ as calculated by a web-based computer tool SNAP in Caucasian panel.²³ We genotyped SNP rs2956540 in the discovery cohort of patients with keratoconus and compared allele frequency with that of unaffected controls. Since at that time, samples from CHS Caucasian controls were not available for genotyping, we imputed their genotypes from an open-source genotype imputation software (HapMap Phase I and II, release #22, NCBI Build 36). We found a positive association with a *P* value of 7×10^{-3} (Table 2). Three additional SNPs in the *LOX* gene were not polymorphic in the replication panel.

Genetic Association Testing of *LOX* Polymorphisms in Families with Keratoconus and Meta-Analysis

We have also performed a family-based association test of the genomewide SNP data using the GWAF package.²¹ As shown in Table 2, positive association with *P* values of 2.7×10^{-3} and 7.7×10^{-4} , respectively, was identified at rs10519694 and

rs2956540 in the families with keratoconus after adjustments for age and sex, thus confirming results in a population-based panel. Meta-analysis of all available patients' samples including two case-control panels and a family panel calculated association *P* values of 4.0×10^{-5} and 2.5×10^{-7} for SNPs rs10519694 and rs2956540, respectively (Table 2).

Sequencing of *LOX* Gene and Identification of Polymorphisms in the Transcribed Sequence

We sequenced the coding sequence, 5' and 3' untranslated regions (UTRs), and exon-intron junctions of *LOX* gene in 16 affected individuals, of which 13 were familial cases and 3 were individual cases from the discovery case-control panel (Table 3). We identified two sequence variants that were further confirmed by sequencing in both directions. The first variant located in the exon 1 of the *LOX* gene corresponds to nonsynonymous G-to-A transition, which results in arginine (R) to glutamine (G) substitution in the *LOX* variant I protein (Fig. 1). Analysis of public SNP databases revealed that this variant corresponds to the known SNP rs1800449, which was first identified as a heritable restriction fragment length polymorphism from normal human lymphocytes.²⁴ The second variant located in the intron 1 of *LOX* gene corresponds to G-to-C substitution in the 5' UTR region of the *LOX* variant II transcript (Fig. 1) and corresponds to rs2288393 in the SNP database. Both sequence variants we identified in the same 3 patients in the heterozygous form (Table 3), thus suggesting that they are in complete disequilibrium. This conclusion was confirmed by a SNAP web-based computer tool, which calculated pairwise linkage disequilibrium of these SNPs with $r^2 = 1.0$. We identified no other sequence changes in *LOX* exons in these patients.

Testing of Transcribed *LOX* Polymorphisms in Case-Control and Family Cohorts

To test for genetic association of transcribed *LOX* polymorphisms rs1800449 and rs2288393 with keratoconus, we genotyped a panel of 919 familial and case-control subjects and performed testing for genetic association. The suggestive signal of association between rs1800449 and keratoconus was identified in the case/control panel with an unadjusted *P* value of 0.07. A similar direction of effect as that of the case/control

FIGURE 1. Locations of SNPs rs1800449 and rs2288393 associated with keratoconus in the lysyl oxidase (*LOX*) gene transcripts.

TABLE 4. Results of Association Testing of Transcribed *LOX* SNP rs1800449 in Case-Control and Familiar Cohorts of Patients with Keratoconus

SNP	Position	MA	Cases/Controls (n = 568)					Families (n = 347)					Meta		
			FA	FU	OR	P	P _A	FA	FU	OR	P	P _A	OR	P	P _A
rs1800449	121,413,208	A	0.16	0.21	0.72	0.07	0.20	0.16	0.20	0.78	0.20	0.12	0.74	<u>0.02</u>	<u>0.05</u>

P_A = P value adjusted for age and sex. Significant P values are underlined.

panel was also found in family-based association testing; however, the P value was not significant (Table 4). Meta-analysis in the combined case/control and family panels revealed association with a P value of 0.02. Pairwise linkage disequilibrium between rs1800449 and SNPs rs10519694 and rs2956540 was found to be low ($r^2 = 0.01$ and 0.2 in Caucasian panel, respectively), indicating that additional functional variants may contribute to the overall association signal in the *LOX* gene.

DISCUSSION

In this study we present results showing the apparent involvement of the *LOX* gene in the pathogenesis of keratoconus, as supported by linkage and association results in families and a positive association in two independent case-control panels. Our group has previously reported a linkage peak with a LOD (logarithm of odds) score of 2.0 in a family with familial keratoconus.⁴ The *LOX* gene is located under this peak. In a follow-up study, we subsequently reported TDT (Transmission Disequilibrium Testing) analysis of SNPs in *LOX* identified preferentially untransmitted haplotype of SNPs rs3792803-rs10519694 to the affected family members with keratoconus with P value of 0.014.²⁴ In previous reports (Li X, et al. *IOVS* 2008;49:ARVO E-Abstract 6090; Bykhovskaya Y, et al. *IOVS* 2011;52:ARVO E-Abstract 3291) and in this report, we have shown that the minor allele A of SNP rs10519694 has a lower frequency in patients with keratoconus in two independent case/control cohorts, with P values as low as 2.3×10^{-3} , and in a family sample with a P value of 2.7×10^{-3} . Moreover, we also found a lower frequency of minor allele G of SNP rs2956540 in the confirmation case/control cohort with a P value of 3.5×10^{-3} and in families with a P value of 7.7×10^{-4} and, after we performed individual genotyping of this SNP, in the discovery case/control panel, with a P value of 2.7×10^{-3} . Highly suggestive evidence of association with meta P values of 2.5×10^{-7} and 4.0×10^{-5} was obtained for *LOX* SNPs rs2956540 and rs10519694, respectively, confirming that common *LOX* variants are associated with keratoconus both in familial and sporadic cases.

The *LOX* gene is composed of seven exons and six introns, distributed through approximately 14.5 kb of genomic DNA.^{5,25} Different transcripts are produced from a single gene, as a consequence of differential use of several polyadenylation signals within the 3' UTR, due to the existence of multiple transcription initiation sites, and by alternative splicing.^{5,25-27} Biological relevance and tissue distribution of these isoforms remain unknown. Associated with the keratoconus transcribed *LOX* polymorphism rs1800449 identified through sequencing analysis, is a G-to-A transition located in the exon 1 of *LOX* gene, which results in a substitution from arginine (Arg) to glutamine (Gln) at codon 158 of *LOX* transcript variant I (accession number NM_002317.5) as annotated in the NCBI database (Fig. 1). This polymorphism is proximal to a propeptide cleavage domain²⁸ and was initially identified from lymphocytes of unrelated, apparently healthy persons.²⁴ Recent studies, however, show that this change may be associated with breast cancer and affects the function of

breast cancer cell lines.²⁹ Polymorphism rs2288393 is located in the exon 2 of the *LOX* gene and constitutes a part of transcript variant II (accession number NM_001178102.1) as shown in Figure 1. Transcript variant I codes for *LOX* isoform 1 preproprotein of 417 amino acids (accession number NP_002308.2), transcript variant II codes for *LOX* isoform 2 with 187 amino acids (accession number NP_001171573.1). We hypothesize that the combination of minor alleles at these SNPs may affect the balance and interaction of the two isoforms in the corneal tissue, which potentially leads to the reduction of cross-linking of collagen fibers in the corneal stroma, biomechanically weakening the cornea and thus making it easier for carriers to develop keratoconus.

A deficiency in lysyl oxidase activity had been found in X-linked recessive human connective tissue disorders: cutis laxa,³⁰ Menkes syndrome,³¹ and Ehlers-Danlos syndrome type V.³² Keratoconus has been identified occasionally in patients with Ehlers-Danlos syndrome type VI,³³ type II,³⁴ and type IV.³⁵ An extensive study of *LOX* enzyme's expression and activity in ocular tissues was recently undertaken and revealed interesting insights into the pathogenesis of some retinal abnormalities.³⁶

Extensive examination of human expressed sequences located in the NCBI Unigene database (<http://www.ncbi.nlm.nih.gov/unigene/>) identified multiple expressed sequence tags (ESTs) matching to the *LOX* gene sequence in a variety of tissues including connective tissue and various parts of the eye including retinal pigment epithelium and fetal eye. We have identified an EST derived from an unamplified human keratoconus cornea library constructed by our group in collaboration with the National Eye Institute³⁷ matching to both *LOX* transcripts I and II. Interestingly, upregulation of *LOX* was identified in the expression microarray study, which compared expression of genes in keratoconus epithelium with that of normal controls.³⁸ Although it is not clear which isoform(s) are in fact upregulated, these results support our hypothesis that variation in *LOX* expression may be responsible for the increased keratoconus susceptibility in individuals carrying certain *LOX* gene variants.

Our findings have implications for a new promising therapy that has been shown to retard the progression of keratoconus: corneal collagen cross-linking. In this treatment the keratoconus cornea is exposed to ultraviolet light after being primed with riboflavin. The interaction between the riboflavin and the UV light increases the corneal collagen cross-links, thus biomechanically strengthening and stiffening the cornea and retarding the progression of the disease.^{6-8,39}

This treatment has the potential to significantly reduce the number of corneal transplants, the only treatment available to treat advanced forms of this disease.¹ As this treatment becomes more commonly accepted, our findings might have particular relevance for this therapy. Incorporation of genetic information will ensure that only "genotypically suitable" patients will undergo the treatment, thus fulfilling the promise of individualized medicine. Testing for *LOX* polymorphisms in patients with keratoconus may further improve the effectiveness and safety of collagen cross-linking treatment by reducing negative outcomes and eliminating nonresponders by identifying them prior to treatment.

Summary

Genetic association between polymorphisms in the lysyl oxidase gene and increased susceptibility to keratoconus have been identified in sporadic and familial cases.

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