Purpose: To determine whether the cornea remodeling-related genes aldehyde dehydrogenase 3A1 (ALDH3A1), lysyl oxidase (LOX), and secreted protein acidic and rich in cysteine (SPARC) were potential susceptibility candidate genes for keratoconus in Korean patients, we investigated the associations of single nucleotide polymorphisms (SNPs) in these three genes in Korean patients with keratoconus.

Methods: Genomic DNA was extracted from blood samples of unrelated patients with keratoconus and healthy control individuals. For screening of genetic variations, all exons from the entire coding regions of the ALDH3A1, LOX, and SPARC genes were directly sequenced to determine the presence of mutations. Control individuals were selected from the general population without keratoconus.

Results: In this study, we detected nine SNPs in ALDH3A1, four SNPs in LOX, and 18 SNPs in SPARC. rs116992290, IVS3-62c>t, rs116962241, and rs2228100 in ALDH3A1 and rs2956540 and rs1800449 in LOX were significantly different between patient and control groups. In the SPARC gene, the distribution of the *G allele of EX10+225 T>G ($p = 0.018$; odds ratio, 1.869) was strongly associated with the risk of keratoconus in the Korean population. In haplotype analysis, C-G of rs2956540-rs2288393 in LOX ($p = 0.046$) and C-C-G and G-G-G of rs60610024-rs2228100-rs57555435 ($p = 0.021$ and $p < 0.001$), G-A of IVS3-62 a>g - rs116962241 in ALDH3A1 ($p = 0.048$) predisposed significantly to keratoconus. After cross-validation consistency and permutation tests, two locus model was the best SNP variations interaction pattern.

Conclusions: Our results suggested that genetic variations in ALDH3A1, LOX, and SPARC genes were associated with a predisposition for keratoconus in Korean individuals. Moreover, variations in ALDH3A1 and LOX may serve as strong biomarkers for keratoconus.

Key Words: ALDH3A1, Keratoconus, LOX, Multifactor dimensional reduction, SPARC

Keratoconus is the clinical diagnosis of corneal thinning and protrusion, which results in corneal steepening, altered refractive power, and reduced vision [1]. The manifestations of keratoconus include noninflammatory stromal thinning, corneal protrusion, Fleischer’s ring, Vogt’s striae, increased nerve fiber visibility, and rupture of Bowman’s layer [2]. This disease is an asymmetric, bilateral disease...
that starts in early adolescence and progresses over 10 to 20 years. The visual outcome varies from mild irregular astigmatism to corneal scarring requiring keratoplasty [1,3].

The pathogenesis of keratoconus is not fully understood; however, the progression of disease is known to be associated with a decrease in the biomechanical strength of the cornea, which is composed of collagen and keratocytes [4-6]. Both genetic predisposition and environmental factors, such as contact lens wearing and eye rubbing, are involved in the pathogenesis of keratoconus [1,7,8]. Histological studies have demonstrated that corneal epithelial cells, stromal keratocytes, and extracellular matrix (ECM) are affected in keratoconus corneas [9-11]. Assuming that all layers and tissues are involved in the pathogenesis of keratoconus [12-16], genes related to corneal remodeling may be potential susceptibility candidate genes in patients with keratoconus.

Therefore, in this study, we evaluated the association of single nucleotide polymorphisms (SNPs) in the aldehyde dehydrogenase 3A1 (ALDH3A1), lysyl oxidase (LOX), and secreted protein acidic and rich in cysteine (SPARC) genes in Korean patients with keratoconus.

Materials and Methods

The study sample included 220 patients with unrelated keratoconus and 150 healthy controls. Written informed consent was obtained from all participants, and study was approved by the Medical Ethics Committee of the Catholic University of Korea (KC14TISI0593). The patients were diagnosed with keratoconus based on the following criteria: (1) symptoms of keratoconus, including the Munson sign, protrusion, Vogt’s striae, corneal thickness, scarring, the Fleischer ring, photokeratoscopy, videokeratography, and refractive errors; and (2) medical history, including age, sex, contact lens use, eye rubbing behavior, systemic disease, atopy, and connective tissue disease. One hundred fifty age-matched control individuals with no history of keratoconus were also enrolled from the Korea Eye Tissue and Gene Bank related to Blindness.

Genomic DNA was extracted from peripheral blood samples using a QIAamp DNA blood kit (Qiagen, Valencia, CA, USA). Polymerase chain reaction (PCR) was performed with 25 ng of genomic DNA as a template in a mixture of PCR buffer, 2.5 mM MgCl2, 200 nM dNTPs, 0.4 pmol of each primer, and 0.75 units of h-Taq polymerase (Enzymomics, Seoul, Korea) (Table 1). For DNA sequencing, amplified DNA was purified using a QIAquick PCR purification kit (Qiagen) and sequenced directly using a BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer’s instructions.

In SNP selection and genotyping, we searched the public domain of the National Center for Biotechnology Information Single Nucleotide Polymorphisms database (NCBI dbSNP) at http://www.ncbi.nlm.nih.gov/snp to identify potentially functional polymorphisms in cell remodeling-related genes. Primers were designed according to the published nucleotide sequence in the ENSEMBL database using Primer3 software for LOX, ALDH3A1, and SPARC (Table 1).

To determine statistically significant differences between the groups by genotyping of SNPs, we used chi-square tests and 2 × 2 and 2 × m Fisher exact tests for the contingency table file. The 2 × 2 contingency tables for each individual allele and the 2 × m contingency tables for each locus were used, where m refers to the number of marker alleles detected in the population. Results with p-values of less than 0.05 were considered statistically significant. The strength of the association was estimated by odds ratio (OR) of risk and 95% confidence intervals (CIs) (JavaStat, http://members.aol.com/johnp71/cstab2x2.html). Haplotype frequencies and linkage disequilibrium measures were estimated using the Haplovew package ver. 4.0 [17]. Haplotyp frequency and associations were calculated with Haplovew ver. 4.0 (http://www.broadinstitute.org/haplovew/haplovew), which uses the expectation maximization algorithm. Haplotype distributions were evaluated by permutation tests on the basis of 10,000 replications to obtain empirical significance.

Potential locus-locus interactions were evaluated using nonparametric MDR software ver. 2.0 alpha (https://www.multifactordimensionalityreduction.org) with risk alleles. Briefly, the multilocus genotypes were pooled into high-risk and low-risk groups, effectively reducing the genotype predictors to one dimension. The new, one-dimensional multilocus-genotype variable was then evaluated for its ability to classify and predict disease status through cross-validation and permutation testing. A detailed explanation on the MDR method has been described elsewhere [18].
| Primers                | bp   |
|-----------------------|------|
| ALDH3A1_4F            | 750  |
| ALDH3A1_4R            | 871  |
| ALDH3A1_6F2           | 871  |
| ALDH3A1_6R2           | 994  |
| ALDH3A1_7F2           | 994  |
| ALDH3A1_7R2           | 994  |
| ALDH3A1_8F            | 656  |
| ALDH3A1_8R            | 656  |
| ALDH3A1_10F           | 761  |
| ALDH3A1_10R           | 761  |
| LOX_Exon 1-1 F1       | 995  |
| LOX_Exon 1-1 R1       | 995  |
| LOX Exon 1-2 F        | 336  |
| LOX Exon 1-2 R        | 336  |
| LOX Exon 2 F          | 543  |
| LOX Exon 2 R          | 543  |
| LOX Exon 3 F1         | 911  |
| LOX Exon 3 R1         | 911  |
| LOX Exon 4 F          | 358  |
| LOX Exon 4 R          | 358  |
| LOX IVS 4 F-1         | 964  |
| LOX IVS 4 R-1         | 964  |
| LOX IVS 4 F1          | 693  |
| LOX IVS 4 R1          | 693  |
| LOX Exon 5 F1         | 936  |
| LOX Exon 5 R1         | 936  |
| LOX Exon 6 F          | 388  |
| LOX Exon 6 R          | 388  |
| SPARC 2F              | 556  |
| SPARC 2R              | 556  |
| SPARC 3F              | 617  |
| SPARC 3R              | 617  |
| SPARC 4F              | 567  |
| SPARC 4R              | 567  |
| SPARC 5F              | 679  |
| SPARC 5R              | 679  |
| SPARC 6F              | 601  |
| SPARC 6R              | 601  |
| SPARC 7F              | 637  |
| SPARC 7R              | 637  |

(Continuing)
Results

The mean ages of patients with keratoconus and normal controls were 28.00 ± 7.75 and 26.83 ± 11.47 years, respectively. The percentages of men were 64.0% in patients with keratoconus and 65.8% in controls. We analyzed nine SNPs in ALDH3A1, four SNPs in LOX1, and 18 SNPs in SPARC (Table 2). Statistically significant genotype and allele frequencies of ALDH3A1, LOX, and SPARC gene variants in patients with keratoconus are listed in Table 3.

Four of nine SNPs in ALDH3A1 were significantly different in the patient and control groups; for rs16992290 (IVS3-193G>a), the frequency of the *g/*g genotype was lower in patients with keratoconus (77.6%) than in the control group (93.4%; \( p = 0.07; \text{OR, } 0.25; 95\% \text{ CI, } 0.075–0.748 \)). The frequency of the *g/*a genotype of rs116992290 was higher in patients with keratoconus (20.0%) than in normal controls (6.6%; \( p = 0.020; \text{OR, } 3.55; 95\% \text{ CI, } 1.145–11.715 \)). The *g allele frequency at rs11699290 was lower in patients with keratoconus (87.6%) than in the control group (96.7%; \( p = 0.003; \text{OR, } 0.24; 95\% \text{ CI, } 0.077–0.700 \)). For IVS3-62c>t, the frequency of the *t/*t genotype was lower in patients with keratoconus (82.2%) than in normal controls (6.6%; \( p = 0.020; \text{OR, } 3.55; 95\% \text{ CI, } 1.145–11.715 \)). The *g allele frequency at rs11699290 was lower in patients with keratoconus (87.6%) than in the control group (96.7%; \( p = 0.003; \text{OR, } 0.24; 95\% \text{ CI, } 0.077–0.700 \)).

Two of three SNPs in LOX were significantly different between keratoconus and normal controls; for rs1800449 (R158Q), the frequency of *A/*A genotype had a lower frequency in patients with keratoconus (1.3%) than in normal controls (7.5%; \( p = 0.002; \text{OR, } 0.16; 95\% \text{ CI, } 0.041–0.597 \)). For rs2956540, the frequency of *c/*c genotype showed a lower frequency in patients with keratoconus (3.7%) than in controls (13.7%; \( p = 0.001; \text{OR, } 0.23; 95\% \text{ CI, } 0.094–0.567 \)), and the *g allele frequency was higher in patients with keratoconus (77.8%) than in controls (60.2%; \( p = 0.011; \text{OR, } 1.56; 95\% \text{ CI, } 1.088–2.236 \)).

One of 19 SNPs in SPARC was significantly different between patients with keratoconus and normal controls; for EX10+225 T>G, the *T/*T genotype frequency was lower in patients with keratoconus (36.7%) than in controls (60.3%; \( p = 0.003; \text{OR, } 0.34; 95\% \text{ CI, } 0.195–0.746 \)), and the *T/*G genotype was higher in patients with keratoconus (63.3%) than in controls (39.7%; \( p = 0.003; \text{OR, } 2.62; 95\% \text{ CI, } 1.340–5.140 \)). The *T allele frequency of EX10+225 T>G was lower in patients with keratoconus (68.3%) than in controls (76.2%; \( p = 0.018; \text{OR, } 0.54; 95\% \text{ CI, } 0.313–0.911 \)).

In haplotype analysis, we identified rs60610024-rs2228100-rs5755435 and IVS3-62 a>g-rs16962241 for...
Table 2. Observed SNPs in ALDH3A1, LOX1, and SPARC genes

| Position | Nucleotide | Amino acid | dbSNPs     |
|----------|------------|------------|------------|
| **ALDH3A1** (9 SNPs) | Exon 4 | IVS3-193g>a | rs116992290 |
| | | IVS3-170c>t | rs887240 |
| | | IVS3-62c>t |  |
| | | IVS3-43g>t |  |
| | | TCA>GCA | S134A | rs887241 |
| | Exon 8 | IVS7-41 g>t |  |
| | | IVS7-29 g>a | rs60610024 |
| | | CCG>GCG | P329A | rs22883100 |
| | Exon 10 | TAC>TAT | Y413Y | rs57555435 |
| **LOX1** (4 SNPs) | Exon 1 | CCG>CAG | R158Q | rs1890449 |
| | Intron 1 | g>c |  |
| | Intron 4 | G>C | rs2956540 |
| | | g>a | rs10519694 |
| **SPARC** (18 SNPs) | Exon 3 | EX3+9A>G | E22E | rs2304052 |
| | Intron 3 | IVS3+36 t>g |  |
| | | IVS3+42 t>c |  |
| | Intron 4 | IVS4+31c>t |  |
| | | IVS4+127 a>g |  |
| | | IVS4+143 g>a |  |
| | | IVS4+153 g>c |  |
| | Intron 5 | IVS4-234 a>c |  |
| | | IVS4-288 t>c |  |
| | Exon 5 | EX5+30 G>A | G80C |
| | Exon 8 | EX8+48 C>T | H211H |
| | Intron 8 | IVS8+26 c>t |  |
| | Intron 9 | IVS8-35 a>g |  |
| | | IVS8-27 g>a |  |
| 3’ UTR | IVS9-53 c>t |  |
| | EX10+58 C>G |  |
| | EX10+212 G>A |  |
| | EX10+225 T>G |  |

SNP = single nucleotide polymorphism; ALDH3A1 = aldehyde dehydrogenase 3A1; LOX = lysyl oxidase; SPARC = secretory protein acidic and rich in cysteine.

ALDH3A1, rs2956540-rs2288393 for LOX, and EX-10+58C>G-IVS9-53c>t, IVS4-234a>c-IVS4+153g>c and IVS4+127a>g-IVS4+31c>t-IVS3+42t>c-IVS3+36t>g-EX-3+9A>G for SPARC (Fig. 1). The G-C (LOX H2) and G-G (LOX H3) haplotypes in LOX (rs2956540-rs2288393) were less frequent in patients with keratoconus than in controls (p = 0.360 and p = 0.058). In ALDH3A1, rs60610024-rs22883100-rs57555435 haplotype (ALDH3A1 H1: C-C-G) was more prevalent in patients with keratoconus than in the control group (p = 0.021), and the C-G-G (ALDH1A1 H2) haplotype was less frequent in patients with keratoconus than in controls (p < 0.001). The IVS3-62 a>g-rs116962241 (ALDH3A1 H5 : C-G) haplotype was more prevalent in patients with keratoconus than in the control.
Table 3. Genotype and allele frequencies of ALDH3A1, LOX, and SPARC genes variants in keratoconus patients

| Gene       | Lead SNP        | Genotypes/alleles | KTCN (%) | CNT (%) | p-value | OR     | 95% CI          |
|------------|-----------------|-------------------|----------|---------|---------|--------|-----------------|
| ALDH3A1    | rs116992290     | g/g               | 77.6     | 93.4    | 0.007   | 0.25   | 0.075<<0.748    |
|            |                 | g/a               | 20.0     | 6.6     | 0.020   | 3.55   | 1.145<<11.715   |
|            |                 | g                 | 2.4      | 0.0     | 0.500   | 18.31  | 0.204<<2526     |
|            |                 | a                 | 0.876    | 0.967   | 0.003   | 0.24   | 0.077<<0.700    |
|            |                 | T                 | 0.124    | 0.033   | 0.003   | 4.14   | 1.428<<12.912   |
|            | rs116962241     | g/g               | 82.6     | 91.2    | 0.035   | 0.46   | 0.223<<0.959    |
|            |                 | g/t               | 16.1     | 7.1     | 0.020   | 2.51   | 1.132<<5.571    |
|            |                 | t/t               | 0.905    | 0.951   | 0.069   | 0.55   | 0.283<<1.056    |
|            | rs2228100       | C/C               | 44.8     | 55.1    | 0.019   | 0.56   | 0.339<<0.910    |
|            |                 | G/G               | 6.3      | 16.9    | <0.001  | 0.23   | 0.107<<0.502    |
|            |                 | G                 | 0.682    | 0.555   | <0.001  | 1.81   | 1.308<<2.490    |
|            |                 | T                 | 0.308    | 0.445   | <0.001  | 0.55   | 0.402<<0.765    |
| LOX        | rs1800449       | GG                | 64.0     | 59.2    | 0.375   | 1.23   | 0.781<<1.924    |
|            |                 | GA                | 34.7     | 33.3    | 0.879   | 0.96   | 0.601<<1.545    |
|            |                 | AA                | 1.3      | 7.5     | 0.002   | 0.16   | 0.041<<0.597    |
|            |                 | G                 | 0.814    | 0.758   | 0.084   | 1.39   | 0.955<<2.024    |
|            |                 | A                 | 0.168    | 0.242   | 0.084   | 0.72   | 0.494<<1.047    |
|            | rs2956540       | gg                | 56.7     | 49.0    | 0.189   | 1.36   | 0.858<<12.167   |
|            |                 | gc                | 39.6     | 37.3    | 0.615   | 0.88   | 0.536<<1.447    |
|            |                 | cc                | 3.7      | 13.7    | 0.001   | 0.23   | 0.094<<0.567    |
|            |                 | g                 | 0.778    | 0.676   | 0.011   | 1.56   | 1.088<<2.236    |
|            |                 | c                 | 0.239    | 0.324   | 0.011   | 0.63   | 0.436<<0.898    |
| SPARC      | EX10+225 T>G    | TT                | 36.7     | 60.3    | 0.003   | 0.38   | 0.195<<0.746    |
|            |                 | TG                | 63.3     | 39.7    | 0.003   | 2.62   | 1.340<<5.140    |
|            |                 | GG                | 0.0      | 0.0     |         |        |                 |
|            |                 | T                 | 0.683    | 0.762   | 0.018   | 0.54   | 0.313<<0.911    |
|            |                 | G                 | 0.317    | 0.189   | 0.018   | 1.87   | 1.097<<3.191    |

ALDH3A1 = aldehyde dehydrogenase 3A1; LOX = lysyl oxidase; SPARC = secretory protein acidic and rich in cysteine; SNP = single nucleotide polymorphisms; KTCN = keratoconus; CNT = control; OR = odds ratio; CI = confidence interval.

In SPARC, no significant results were observed among haplotypes (Table 4). Interaction between LOX, ALDH3A1, and SPARC variations in relation to the risk of keratoconus was evaluated by non-parametric MDR method. Table 5 shows the results of cross validation consistency (CVC), accuracy and OR (95% CI) obtained
Table 4. Haplotype analysis of LOX, ALDH3A1, and SPARC genes in Korean keratoconus patients

| Gene     | Haplotype                     | Case  | Control | Chi-square | p-value |
|----------|-------------------------------|-------|---------|------------|---------|
| **LOX**  | rs2956540-rs2288393           |       |         |            |         |
|          | LOX_H1 : C-G                  | 0.762 | 0.693   | 3.999      | 0.046   |
|          | LOX_H2 : G-C                  | 0.133 | 0.158   | 0.838      | 0.360   |
|          | LOX_H3 : G-G                  | 0.101 | 0.148   | 3.597      | 0.058   |
| **ALDH3A1** | rs60610024-rs2228100-rs57555435 |       |         |            |         |
|          | ALDH3A1_H1 : C-C-G            | 0.569 | 0.477   | 5.33       | 0.021   |
|          | ALDH3A1_H2 : C-G-G            | 0.303 | 0.441   | 13.187     | <0.001  |
|          | ALDH3A1_H3 : A-C-T            | 0.089 | 0.055   | 2.633      | 0.105   |
|          | ALDH3A1_H4 : A-C-G            | 0.031 | 0.026   | 0.144      | 0.704   |
|          | IVS3-62 a>g - rs116962241     |       |         |            |         |
|          | ALDH3A1_H5 : G-A              | 0.9    | 0.945   | 3.922      | 0.048   |
|          | ALDH3A1_H6 : A-C              | 0.089 | 0.05    | 3.27       | 0.071   |
| **SPARC** | EX10+212G>A_EX10+58C>G       |       |         |            |         |
|          | SPARC H1 : C-C                | 0.651 | 0.654   | 0.003      | 0.959   |
|          | SPARC H2 : G-T                | 0.349 | 0.346   | 0.003      | 0.959   |
|          | IVS4-234a>c_IVS4+153g>c       |       |         |            |         |
|          | SPARC H3 : C-G                | 0.904 | 0.926   | 0.499      | 0.479   |
|          | SPARC H4 : G-C                | 0.077 | 0.074   | 0.013      | 0.908   |
|          | IVS4+127a>g_IVS4+31c>t_IVS3+42t>c_IVS3+36c>g_EX3+9A>G |       |         |            |         |
|          | SPARC H5 : A-C-T-T-A          | 0.512 | 0.506   | 0.01       | 0.919   |
|          | SPARC H6 : G-T-T-G-A          | 0.359 | 0.327   | 0.369      | 0.544   |
|          | SPARC H7 : G-T-C-T-G          | 0.088 | 0.093   | 0.019      | 0.889   |
|          | SPARC H8 : G-T-T-T-A          | 0.035 | 0.074   | 2.433      | 0.119   |

LOX = lysyl oxidase; ALDH3A1 = aldehyde dehydrogenase 3A1; SPARC = secretory protein acidic and rich in cysteine.

Fig. 1. Haplotype structure of single nucleotide polymorphisms (SNPs) in (A) aldehyde dehydrogenase 3A1 (ALDH3A1), (B) lysyl oxidase (LOX), and (C) secretory protein acidic and rich in cysteine (SPARC). We estimated the pairwise linkage disequilibrium by calculating pairwise D' and r\(^2\) (D' > 0.70, r\(^2\) > 0.80). The images were generated with the Haploview software pack.
LOX, ALDH3A1, and SPARC genes interactions with overall keratoconus risk based on MDR analysis

| Model                                                                 | Bal.Acc.CV training | Bal.Acc.CV testing | CVC | p-value | Testing OR (95% CI) |
|----------------------------------------------------------------------|---------------------|--------------------|-----|---------|---------------------|
| ALDH3A1_rs2228100                                                    | 0.575               | 0.538              | 8/10| 0.018   | 1.895 (1.113 to 3.229) |
| LOX_rs2956540/ALDH3A1_rs2228100                                     | 0.634               | 0.633              | 10/10| <0.001 | 3.164 (1.881 to 5.324) |
| LOX_rs2956540/ALDH3A1_rs116962241/ALDH3A1_rs2228100                 | 0.649               | 0.581              | 5/10| <0.001 | 3.541 (2.103 to 5.962) |
| LOX_rs1800449/ALDH3A1_rs116992290/ALDH3A1_rs3744694/ALDH3A1_rs2228100 | 0.672               | 0.658              | 9/10| <0.001 | 4.563 (2.680 to 7.769) |
| LOX_rs1800449/LOX_rs2956540/ALDH3A1_rs116962241/ALDH3A1_rs2228100    | 0.684               | 0.613              | 9/10| <0.001 | 5.054 (2.956 to 8.642) |

LOX = lysyl oxidase; ALDH3A1 = aldehyde dehydrogenase 3A1; SPARC = secretory protein acidic and rich in cysteine; CVC = cross-validation consistency; OR = odds ratio; CI = confidence interval. *1000-fold permutation test.

Discussion

Keratoconus is multifactorial disease with complex etiology, and some genetic conditions including inflammatory bowel disease, familial Mediterranean fever and Down syndrome, are known to be associated with keratoconus [19-21]. However, isolated keratoconus without any genetic association is far more frequent, and previous studies have focused on the identification of genes related to this type of keratoconus [1,2,22]. In the present study, we investigated the impact of corneal remodeling genes, including ALDH3A1, LOX, and SPARC polymorphisms, on the risk of keratoconus in a sample Korean population. LOX and SPARC are localized on chromosomes 5q23.2 and 5q31.3-q32, respectively [23,24]. Because this region shows possible linkage in familial keratoconus, both genes were assumed to be candidate genes in keratoconus.

LOX is one of the most extensively studied genes in the field of keratoconus genetic analysis [25-27]. LOX is expressed in the cornea, vitreous, iris/ciliary body, lens, choroid/retinal pigment epithelium, and retina and initiates the crosslinking of two basic components of the ECM, which includes collagens and elastin, by catalyzing oxidative deamination of the epsilon-amino group in certain lysine and hydroxylysine residues [26,28]. Moreover, LOX protein affects the assembly, tensile strength, and mechanical stability of collagen fibrils. Previous genotyping studies have confirmed the effects of the SNP rs2956540 in LOX in European, Chinese, and Iranian populations and in a meta-analysis [26,29-31]. Our study showed the rs2956540 of LOX had an exceptionally high odds ratio in patients with keratoconus and also could be a genetic biomarker in unrelated Korean patients with keratoconus.

SPARC encodes secreted protein acidic and rich in cysteine/osteonectin/BM40, a matrix-associated protein that elicits changes in cell shape, inhibits cell-cycle progression, and influences the synthesis of the ECM [25,32-34]. SPARC is found in the ECM and is predominantly expressed during embryogenesis and in adult tissues undergoing remodeling or repair. SPARC plays a role in cell-cell and cell-matrix interactions, differentiation, ECM production and organization, wound healing, and angiogenesis [34]. The ECM-related function of SPARC and the observation of a region near to LOX suggested that SPARC may have a role in the pathogenesis of keratoconus [25]. Previous findings have shown various outcomes related to SPARC in genotyping in patients with keratoconus, suggesting that such polymorphisms are rare rather than strong candidates for unrelated keratoconus-susceptibility genes [23,25,35].

ALDH3A1 encodes aldehyde dehydrogenase 3 family, member A1, which is localized on chromosome 17p11.2 [36]. ALDH3A1 is a nuclear protein expressed in the corneal epithelium and stroma that has roles in cell cycle regulation and corneal homeostasis by modulating prolifera-
tive and differentiation programs [36-38]. ALDH3A1 also has a protective effect on cells during environmental stressors, and previous studies have found that ALDH3A1 is upregulated in keratoconus corneal stroma, as identified by two-dimensional-difference gel electrophoresis. We found four SNPs in ALDH3A1 in patients with keratoconus, including rs116992290 (IVS3-193g>a), IVS3-62c>t, rs116962241 (IVS3-43g>t), and rs2228100 (P329A). In the human cornea, ALDH3A1 was found in the epithelium and stroma, but not in the endothelium [36-38]. Additionally, high expression of ALDH3A1 in mouse epithelium was altered after light exposure, suggesting that ALDH3A1 may play a role in the constantly exposed and changing cornea to maintain corneal homeostasis [36].

MDR analysis detected significant high order interactions for Keratoconus in our study. The most significant finding was the association between the LOX_rs2956540/ALDH3A1_rs2228100 and Keratoconus-locus by MDR analysis. And compared to the single SNP effect or the haplotype combined effect of LOX and ALDH3A1, a greater odds ratio for the best model with two-locus indicated that a synergistic interaction between the two SNPs was more strongly associated with keratoconus. And 4 SNPs (LOX_rs1800449/ALDH3A1_rs116992290/ALDH3A1_rs3744694/ALDH3A1_rs2228100; CVC 9/10; p < 0.001; OR, 4.563) and 5 SNPs (LOX_rs1800449/LOX_rs2956540/ALDH3A1_rs116962241/ALDH3A1_rs3744694/ALDH3A1_rs2228100; CVC 9/10; p < 0.001; OR, 5.054) including rs2956540 and rs2228100 were associated with a significantly increased risk in Keratoconus. It suggests that it will help to better understand the complex genetic basis of keratoconus. Our results all indicate that a combination of biomarkers provides a better prediction of the risk of keratoconus.

In conclusion, our study results supported that genetic variations in ALDH3A1, LOX, and SPARC genes may be associated with a predisposition for keratoconus in Koreans. Additionally, we demonstrated that rs2228100 of ALDH3A1 gene and rs2956540 in the LOX gene may serve as a genetic biomarker for keratoconus. Further investigations of ALDH3A1, LOX, and SPARC polymorphisms are needed in individuals of different ethnicities and from different countries. Also further study seems necessary to elucidate the role of genetic factors in the development of Keratoconus disease.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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