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Original Article

Association with Corneal Remodeling Related Genes, *ALDH3A1*, *LOX*, and *SPARC* Genes Variations in Korean Keratoconus Patients

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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*

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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 keraconus 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 $MgCl_2$, 200 nM dNTPs, 0.4 pmol of each primer, and 0.75 units of h-Taq polymerase (Enzynomics, 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/ctab2x2.html). Haplotype frequencies and linkage disequilibrium measures were estimated using the Haploview package ver. 4.0 [17]. Haplotype frequencies and associations were calculated with Haploview ver. 4.0 (http://www.broadinstitute.org/haploview/haploview), 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].

Table 1. Primers for single nucleotide polymophisms analysis

	Primers	bp
ALDH3A1_4F	cctctctccctttctgctt	750
ALDH3A1_4R	gagagggcagctgctaagaa	
ALDH3A1_6F2	cagaacccgtatctgcacct	871
ALDH3A1_6R2	tagctcactgcagcctcaaa	
ALDH3A1_7F2	ttgagaccagcctgggtaac	994
ALDH3A1_7R2	gacccgagatctgtctccag	
ALDH3A1_8F	cccgagtttgtcaaggagac	656
ALDH3A1_8R	ctcattcagtgccctcaggt	
ALDH3A1_10F	cctatttcatggaggcctga	761
ALDH3A1_10R	AAGGGGTGGAGACTTGGAAT	
LOX_Exon 1-1 F1	ccccagattaagccagtgtg	995
LOX_Exon 1-1 R1	ACTGAGCGCAGGAACTTCTC	
LOX Exon 1-2 F	CCGTCACTGGTTCCAAGCTG	336
LOX Exon 1-2 R	ACGTCGAGAAGCCACATAGC	
LOX Exon 2 F	CCAGCTATGTGGCTTCTCGAC	543
LOX Exon 2 R	ACTTCCCAGCTCTTGTCCC	
LOX Exon 3 F1	tgcatcactcacaccattga	911
LOX Exon 3 R1	tgggcttcagattttccatt	
LOX Exon 4 F	ATTTGGTCTCAATTTTAATGTG	358
LOX Exon 4 R	ATGCTATTTAATGCTAACTAACGG	
LOX IVS 4 F-1	tgatggcttgatgatccaaa	964
LOX IVS 4 R-1	gggggaaccagaagtgctat	
LOX IVS 4 F1	ctgctcttcccaaatcaagc	693
LOX IVS 4 R1	tgtggcaggaacaatcgtaa	
LOX Exon 5 F1	ttacgattgttcctgccaca	936
LOX Exon 5 R1	atcaagcagggaagggattt	
LOX Exon 6 F	AACGTCTCCAGAGTTTAACCA	388
LOX Exon 6 R	GCATACCATTTTCTGCCTTTG	
SPARC 2F	ggatttctggtaggggtggt	556
SPARC 2R	accacccctaccagaaatcc	
SPARC 3F	cagtgtcatcccctctggat	617
SPARC 3R	gaaggtttgggaagcattca	
SPARC 4F	ctttccctaacacccctggt	567
SPARC 4R	cagggcaaagagctatgagg	
SPARC 5F	ttcaatggagacccaggaac	679
SPARC 5R	ggaacctgatggtgctgttt	
SPARC 6F	gactcagtcatgcctctgct	601
SPARC 6R	ttccctgatgttgaccttcc	
SPARC 7F	agcttcaaacacctgccagt	637
SPARC 7R	ctccaaaggcaggaagagaa	

(Continuing)

Table 1. Continued

	Primers	bp
SPARC 8F	cttcgccaggtgattttgat	561
SPARC 8R	tttctttgtcccaggtccac	
SPARC 9F	atggccatctcctcctcttt	616
SPARC 9R	gtgctaacgcttgaggaagg	
SPARC 10F	ggcagcgtgtgtaagagaca	629
SPARC 10R	GCCAAGACCCTGAAATGAAA	

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

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 rs116992290 (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%; $p = 0.07$; OR, 0.25; 95% 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%; $p = 0.020$; OR, 3.55; 95% CI, 1.145–11.715). The *g allele frequency at rs116992290 was lower in patients with keratoconus (87.6%) than in the control group (96.7%; $p = 0.003$; OR, 0.24; 95% 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 the control group (91.2%; $p = 0.028$; OR, 0.45; 95% CI, 0.217–0.931). The *t/*c genotype frequency at IVS3-62c>t was higher in patients with keratoconus (16.5%) than in controls (8.0%; $p = 0.028$; OR, 2.30; 95% CI, 1.003–4.925). For rs116962241 (IVS3-43g>t), the frequency of the *g/*g genotype was lower in patients with keratoconus (82.6%) than in controls (91.2%; $p = 0.035$; OR, 0.46; 95% CI, 0.223–0.959), and the frequency of *g/*t genotype was higher in patients with keratoconus (16.1%) than in controls (7.1%; $p = 0.020$; OR, 2.51; 95% CI, 1.132–5.571). The frequency of the *C/*C genotype of rs2228100 (P329A) was higher in patients with keratoconus (44.8%) than in controls (28.0%; $p = 0.002$; OR, 2.09; 95% CI, 1.297–3.361). The frequency of the *G/*G genotype was lower in patients with keratoconus (6.3%) than in controls (16.9%; $p < 0.001$; OR, 0.23; 95% CI, 0.107–0.502), and the frequency of *C/*G genotype was lower in patients with keratoconus (49.0%) than in controls (55.1%; $p = 0.019$; OR, 0.56; 95% CI, 0.339–0.901). Finally, the *C allele frequency of rs2228100 was higher in patients with keratoconus (68.2%) than in controls (59.2%; $p < 0.001$; OR, 1.81; 95% CI, 1.308–2.490).

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%; $p = 0.002$; OR, 0.16; 95% 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%; $p = 0.001$; OR, 0.23; 95% CI, 0.094–0.567), and the *g allele frequency was higher in patients with keratoconus (77.8%) than in controls (60.3%; $p = 0.011$; OR, 1.56; 95% 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%; $p = 0.003$; OR, 0.34; 95% CI, 0.195–0.746), and the *T/*G genotype was higher in patients with keratoconus (63.3%) than in normal controls (39.7%; $p = 0.003$; OR, 2.62; 95% 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%; $p = 0.018$; OR, 0.54; 95% CI, 0.313–0.911).

In haplotype analysis, we identified rs60610024-rs2228100-rs57555435 and IVS3-62 a>g-rs116962241 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		rs116962241
		TCA>GCA	S134A	rs887241
	Exon 8	IVS7-41 g>t		
		IVS7-29 g>a		rs60610024
		CCG>GCG	P329A	rs2228100
	Exon 10	TAC>TAT	Y413Y	rs57555435
LOX1 (4 SNPs)	Exon 1	CGG>CAG	R158Q	rs1800449
	Intron 1	g>c		rs2288393
	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		rs1978707
		IVS4+127 a>g		
		IVS4+143 g>a		
		IVS4+153 g>c		
	Intron 5	IVS4-234 a>c		
		IVS4-228 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-rs2228100-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
			2.4	0.0	0.500	18.31	0.204<<2526
		g	0.876	0.967	0.003	0.24	0.077<<0.700
		a	0.124	0.033	0.003	4.14	1.428<<12.912
	IVS3-62c>t	c/c	1.2	0.9	0.702	1.55	0.160<<15.115
		c/t	16.5	8.0	0.028	2.30	1.003<<4.925
		t/t	82.2	91.2	0.028	0.45	0.217<<0.931
		c	0.095	0.049	0.069	1.83	0.947<<3.528
		t	0.905	0.951	0.069	0.55	0.283<<1.056
	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	1.2	1.8	0.779	0.77	0.127<<4.696
		g	0.907	0.947	0.069	0.55	0.283<<1.056
		t	0.093	0.053	0.069	1.83	0.947<<3.528
	rs2228100	C/C	44.8	28.0	0.002	2.09	1.297<<3.361
		C/G	49.0	55.1	0.019	0.56	0.339<<0.910
		G/G	6.3	16.9	<0.001	0.23	0.107<<0.502
		C	0.682	0.555	<0.001	1.81	1.308<<2.490
		G	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	0.186	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; KTCM = keratoconus; CNT = control; OR = odds ratio; CI = confidence interval.

group (p = 0.046). 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

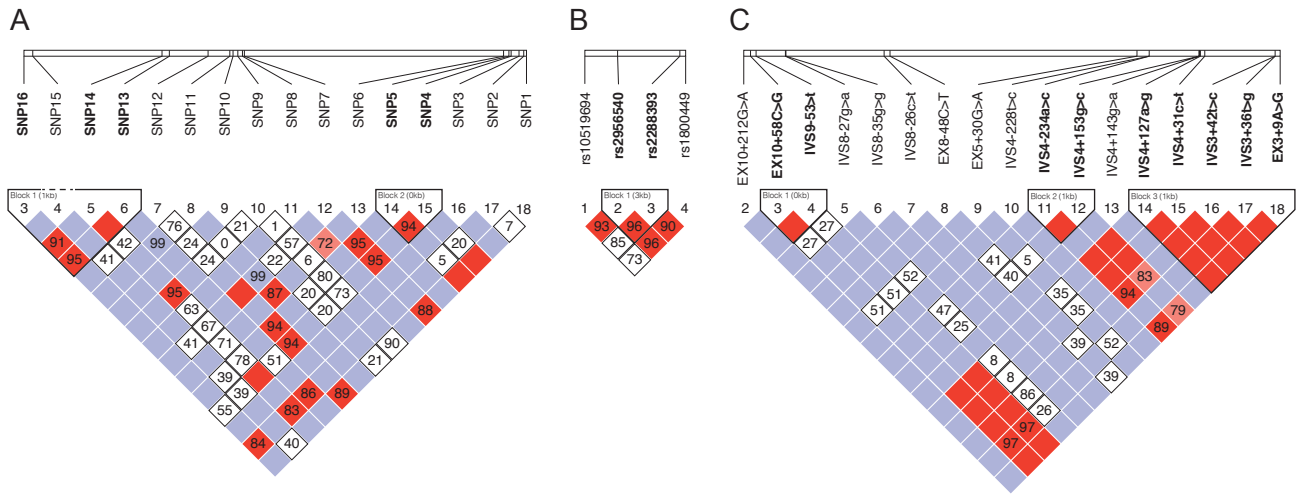

Fig. 1. Haplotype structure of single nucleotide polymorphisms (SNPs) in (A) aldehyde dehydrogenase 3A1 (*ALDH3A1*), (B) lysyl oxidase (*LOX*), and (C) secreted protein acidic and rich in cysteine (*SPARC*). We estimated the pairwise linkage disequilibrium by calculating pairwise D' and r^2 (D' > 0.70,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

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 H14 : G-C	0.077	0.074	0.013	0.908
	IVS4+127a>g_IVS4+31c>t_IVS3+42t> c_IVS3+36t>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.

Table 5. *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_rs3744694/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.

from MDR analysis. LOX_rs2956540/ALDH3A1_ rs2228100 was the best model of SNP interaction for Keratoconus risk (CVC; 10/10, accuracy 0.634, $p \leq 0.001$).

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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