Substitution at *IL1RN* and Deletion at *SLC4A11* Segregating with Phenotype in Familial Keratoconus

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Purpose. Keratoconus (KTCN) is a thinning and anterior protrusion of the cornea that results in altered refractive powers and loss of visual acuity. Despite numerous studies, the reasons for development and progression of KTCN remain unknown. Genetic studies have led to identification of several loci linked with KTCN, including a locus in one multigenerational Ecuadorian family. The purpose of this study was to identify sequence variants in candidate genes segregating with the KTCN phenotype in another Ecuadorian family.

Methods. Nonparametric linkage analysis was performed in Ecuadorian family KTCN-019. Candidate genes *IL1A*, *IL1B*, *IL1RN*, and *SLC4A11* were selected and examined in this family by direct sequencing of all exons, promoters, and intron-exon junctions.

Results. Two novel suggestive loci were identified in 2q13-q14.3 and 20p13-p12.2. Screening of the candidate genes revealed 66 sequence variants, including five novel variants, in both coding and noncoding regions. The substitution c.214+242C > T in the *IL1RN* gene was observed in all affected individuals and three apparently unaffected family members. The novel deletion of 54 nucleotides in position c.2558+149_2558+203 in *SLC4A11* was observed in all patients but one, as well as two healthy individuals and one person with an unknown phenotype.

Conclusions. The analyses of selected genes have led to identification of numerous sequence variants in the examined Ecuadorian family. Both substitution c.214+242C > T in *IL1RN* and novel deletion c.2558+149_2558+203del54 in *SLC4A11* were observed significantly more frequently in family members with KTCN ($P = 0.004525$ and $P = 0.00761$, respectively), suggesting involvement of these two genes in KTCN etiology in the studied family. (*Invest Ophthalmol Vis Sci.* 2013;54:2207–2215) DOI:10.1167/iovs.13-11592

Keratoconus (KTCN, OMIM 148300) is a thinning and anterior protrusion of the cornea that results in altered refractive powers and loss of visual acuity. The prevalence of the disease is estimated to be 1:2000 in the general population.[1] Although most diagnosed KTCN cases are sporadic, patients with positive family history are also observed.[2] The frequency of familial KTCN is ranged between 6% and 26% of KTCN cases.[3–5] Despite numerous studies, the reasons of development and progression of this disorder remain elusive. Genetic studies of KTCN families have led to identification of several significant and suggestive loci linked with KTCN.[2] To date, only one locus, 5q21.2, has been described in two different populations.[6,7] Although the majority of genetic studies were performed for familial forms of KTCN, unrelated individuals with KTCN were also examined in single-gene analyses[8] and whole-genome studies.[9,10] One of the most widely studied genes in KTCN etiology is *VSX1*. Mutations in *VSX1* have been described as related to the KTCN phenotype.[11–13] However, numerous studies, have not confirmed these findings.[14–17] The second candidate gene analyzed in KTCN is *SOD1*. Although some disease-causing mutations in *SOD1* have been indicated as related with KTCN,[18] this relationship has not been confirmed in other populations.[15,19,20]

Among the environmental factors, frequent eye rubbing[21] and contact lens wearing[22] are mentioned. Moreover, in some reports, coexistence of KTCN with atopy is presented.[23–25] In numerous studies, an oxidative stress, as a factor influencing the development of KTCN is also reported.[26–28] Because the cornea is the first part of the eye exposed to UV light, UV light is considered as a possible cause of the oxidative stress.[29] As a result of oxidative stress, the epithelial cells could be stimulated to release IL-1,[30] which is critical for the inflammatory process and the broad spectrum of action. Wilson et al.[31] proposed participation of IL-1 in apoptosis of the corneal cells, which might lead to the development of KTCN and other diseases of the cornea.

IL-1α and IL-1β (encoded by *IL1A* and *IL1B*, respectively) are cytokines responsible for the control of the proinflammatory response, immune response, and hematopoiesis,[32] whereas IL-1 receptor antagonist (IL-1Ra) modulates the effects of IL-1α and IL-1β.[33] The genes of both interleukins together with the gene encoding IL-1Ra protein (*IL1RN*) are located in the human genome in a cluster on 2q13 chromosome. Association analysis of genes *IL1A*, *IL1B*, and *IL1RN* in unrelated individuals from

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the Korean population revealed the existence of two polymorphisms in the promoter of the *IL1B* gene (rs16944 and rs1143627), correlating with the KTCN phenotype.[8]

Sodium bicarbonate transporterlike protein 11 (SLC4A11) is a member of the SLC4 family of bicarbonate transporters.[34,35] This protein, encoded by the *SLC4A11* gene on the 20p13 chromosome, functions as an electrogenic Na^+-coupled borate cotransporter.[34] A recent study indicated that failure in SLC4A11 function leads to apoptosis.[36] It is known that apoptosis can be related to KTCN.[37]

In this study, we report evidence of linkage between two novel suggestive loci and KTCN in a large, multigenerational Ecuadorian family. We also describe a detailed analysis of four candidate genes, *IL1A*, *IL1B*, *IL1RN*, and *SLC4A11*, localized at the newly identified loci, which may be involved in KTCN etiology.

Methods

Subjects

Twenty-one members of an Ecuadorian KTCN-019 family, including nine affected, nine unaffected, and three individuals of unknown phenotype, were identified and examined in the Hospital Metropolitano in Quito, Ecuador. All of the individuals underwent a complete ophthalmic assessment, including visual acuity, intraocular pressure, biomicroscopic evaluation, and fundus examination with dilation. A topographic study (Humphrey Atlas Topograph; Carl Zeiss Meditec, Jena, Germany) with a computer-assisted videokeratoscope was performed in all affected members of family KTCN-019. The detailed ascertainment and examination processes have been previously described. Briefly, the diagnosis of KTCN was established in subjects with at least two of the following topographic characteristics: (1) the curvature of the cornea more than 47 diopters (D) (normal, ~43 D), (2) acentric or irregular corneal video keratography shapes, and (3) inferior–superior value differences of 3 D, 3 mm below and above the center, as have been previously described.[15] The 93 DNA samples from affected and unaffected individuals from another 19 Ecuadorian KTCN families and DNA samples from 22 unrelated healthy Ecuadorian individuals were used as controls. In accordance with the Declaration of Helsinki, the possible consequences of the study were explained and written informed consent was obtained from all participating individuals. The research protocol was approved by the institutional review board at Poznan University of Medical Sciences in Poland.

Linkage Analysis and Loci Identification

A genome-wide screen was performed by genotyping the KTCN-019 family with fluorescently labeled microsatellite markers, as previously described.[15] PEDSTATS v. 0.6.10[38] (http://www.sph.umich.edu/csg/abecasis/PedStats/) was used to identify potential Mendelian inconsistencies. Because the model of inheritance for KTCN is unknown, whole-genome multipoint nonparametric linkage (NPL) analyses were performed with the SimWalk2 v.2.91 program (http://watson.hgen.pitt.edu/docs/simwalk2.html).[39,40] Mega2 v.4.5.1[41] (http://watson.hgen.pitt.edu/mega2.html) was used to construct all the input files for the SimWalk2 program. SimWalk2 contains five NPL statistics: BLOCKS, MAX-TREE, ENTROPY, NPL PAIR, and NPL ALL for each marker. BLOCKS is the most powerful at detecting linkage to a recessive trait, MAX-TREE was designed for traits best modeled by dominant inheritance, ENTROPY is a measure of the entropy of the alleles among the affected cases, and NPL PAIR and NPL ALL are the most powerful at detecting linkage to an additive trait. The Rutgers Combined Linkage-Physical Map was used to estimate loci genetic map distances.[42]

Haplotypes were reconstructed using the SimWalk2 v.2.91[39,43] and visualized with HaploPainter v.1.043 program (http://haplopainter.sourceforge.net/).[44]

Candidate Gene Screening

Oligonucleotide primer pairs for amplification of all coding regions, intron-exon junctions, and UTRs of *IL1A*, *IL1B*, *IL1RN*, and *SLC4A11* genes were designed with the Primer3 v. 0.4.0 tool[45] (http://frodo.wi.mit.edu/) (see Supplementary Material and Supplementary Table S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.13-11592/-/DCSupplemental). PCR amplifications were performed with *Taq* DNA Polymerase (Fermentas, Inc., Glen Burnie, MD). PCR products were purified with ExoSAP-IT (USB Corporation, Cleveland, OH) and sequenced in both directions with BigDye Terminator v3.1 Chemistry (Applied Biosystems, Inc. [ABI], Foster City, CA). Sequencing results were visualized on the 3730xl DNA Analyzer (ABI). Fragments were compared with the reference sequences of *IL1A*, *IL1B*, *IL1RN*, and *SLC4A11* genes (GenBank accession numbers for the mRNA NM_000575.3, NM_000576.2, NM_173841.2, and NM_001174089.1, respectively) using Sequencher 5.0 software (Gene Codes Corporation, Ann Arbor, MI).

Prediction of Effect of Amino Acid Substitutions on Protein Function

The potential impact of nonsynonymous amino acid substitutions on the IL-1α protein structure and function was assessed with PolyPhen-2 v.2.1.0 (http://genetics.bwh.harvard.edu/pph2/) and SIFT v.4.0.3b (http://sift.jcvi.org/). The PolyPhen-2 algorithm predicts which missense changes affect structure and function of the protein. PolyPhen-2 uses Position-Specific Independent Counts (PSIC) software to assign profile scores. These scores are the likelihood of a given amino acid occurring at a specific position, compared with likelihood of this amino acid occurring at any position (background frequency).[46]

The SIFT analytic tool evaluates conserved positions and calculates a score for the amino acid change at a particular position. A score greater than 0.05 is considered as tolerated for the protein structure.[47]

Analysis of Possible Splicing Alteration

Human Splicing Finder v. 2.4.1 (HSF)[48] (http://www.umd.be/HSF/) was used to predict possible effects of observed sequence variants on splicing signals and to identify splicing motifs. This program includes several matrices (MaxEnt, ESEfinder, and PESX)[48] to analyze splice sites and splicing silencers and enhancers. The consensus values (CVs) for both wild-type and sequence variant and the variations of the consensus values (ΔCV) obtained for both wild-type and variant motifs were analyzed. The sequence variants that showed predictions with CVs higher than 70 to 80 and/or ΔCV reductions of at least 10% are likely to affect splicing.[48]

Results

Linkage Analysis and Loci Identification

A genome-wide screen of family KTCN-019 indicated two distinct loci. Table 1 displays NPL multipoint scores for five statistics: BLOCKS, MAX-TREE, ENTROPY, NPL PAIR, and NPL ALL in family KTCN-019 for chromosomes 2 and 20. Maximum NPL PAIR score for chromosome 2 was obtained at marker D2S160 (NPL score = 2.395). Additionally, marker D2S160 had the highest NPL score for MAX-TREE statistics (NPL score = 2.135), which is most powerful for a dominant model. The haplotype inherited by all affected individuals and two unaffected members of KTCN-019, was surrounded by markers D2S293 and D2S2271 (Figure, red bars). In addition, for chromosome 20, the maximum NPL PAIR score was obtained at marker D20S889 (NPL score = 2.409). The same marker had the highest NPL score for BLOCKS statistics (NPL score = 2.699), the most powerful tool at detecting linkage to a recessive trait. However, after haplotype reconstruction of

Table 1. Multipoint Nonparametric Analysis Results of Selected STR Marker Loci on Chromosomes 2 and 20 in Family KTCN-019

Marker Names	Genetic Position, cM*	Physical Position, Start, bp†	Bloks	Max-Tree	Entropy	NPL Pair	NPL All
D2S2216	109.0411	88,309,641	0.249	0.388	0.722	0.657	0.738
D2S2264	113.0712	102,319,557	0.247	1.316	1.157	1.316	1.541
D2S293	116.7009	107,173,714	0.252	1.313	1.156	1.303	1.541
D2S160	121.3307	112,898,435	1.230	2.135	2.283	2.395	2.714
D2S347	131.8409	124,149,810	1.216	2.072	2.289	2.345	2.786
D2S2271	135.9404	127,999,729	0.255	1.094	1.04	1.125	1.22
D2S151	153.6401	147,686,514	0.255	0.061	0.176	0.064	0.094
D20S117	0.0000	555,092	2.405	0.709	2.466	2.181	1.748
D20S906	3.0300	1,405,567	2.512	0.706	2.511	2.262	1.792
D20S842	6.2196	2,586,204	2.656	0.708	2.667	2.358	1.771
D20S889	8.1899	3,846,953	2.699	0.703	2.699	2.409	1.804
D20S882	13.5502	5,535,098	2.439	0.708	2.627	2.302	1.692
D20S846	17.8597	6,664,933	2.076	0.713	2.455	2.202	1.632
D20S115	19.9696	7,559,867	1.824	0.719	2.252	2.054	1.552
D20S186	30.8190	11,423,795	1.217	0.724	1.72	1.563	1.2
D20S898	35.2890	15,176,211	0.506	0.722	1.144	1.088	1.114

* The Rutgers Combined Linkage-Physical Map of The Human Genome.

† National Center for Biotechnology Information (NCBI) build 37.1 genome assembly.

locus 20p13-p12.2, a recessive model of inheritance was not confirmed.

Candidate Gene Screening

IL1A, *IL1B*, and *IL1RN* genes from locus 2q13-q14.3, and *SLC4A11* from locus 20p13-p12.2, were selected for sequencing analysis. All results are presented in Table 2. Ten sequence variants were detected in *IL1A* gene, including one novel c.-982G > A substitution, one known nonsynonymous change c.340G > T (Ala114Ser), and one insertion c.*924_*925insTTCA. Ten sequence variants were identified in *IL1B*, including one novel variation c.99+54G > T and nine known nucleotide substitutions. Among these variants, c.315C > T change results in synonymous substitution Phe105Phe.

Analysis of *IL1RN* gene revealed 26 sequence changes, including one novel c.*480_481delT deletion. Two observed variants, c.180T > C and c.399T > C, cause synonymous amino acid changes, Ala60Ala and Ser133Ser, respectively. The c.214+242C > T substitution was observed more frequently in patients than in healthy individuals in the KTCN-019 family ($P = 0.004525$). This variant was also detected in other KTCN families ($P = 0.54$) and in 10 among 22 healthy unrelated Ecuadorian individuals.

Sequencing of *SLC4A11* has led to identification of 20 sequence variants, including 17 nucleotide substitutions and three sequence length changes: c.292-118_292-

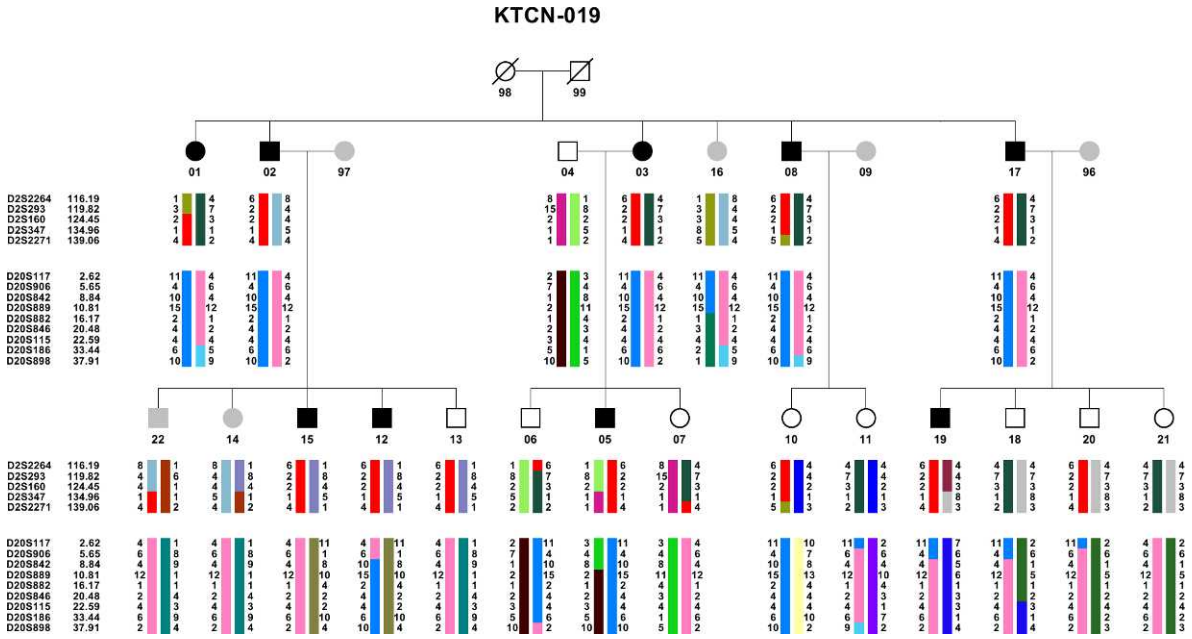

Figure. Pedigree of family KTCN-019 and haplotype analysis. *Black-filled symbols* indicate individuals with KTCN, *gray-filled symbols* indicate individuals with unknown phenotype (14,16, and 22) or individuals without DNA collected (96–99), whereas the *open symbols* indicate unaffected individuals. *Top*: The haplotype at locus 2q12-q14.3. *Bottom*: The haplotype at locus 20p13-p12.2. Haplotype regions in different colors indicate pattern of inheritance.

Table 2. Sequence Variants Found in *IL1A*, *IL1B*, *IL1RN*, and *SLC4A11* Genes

			KTCN-019							Other Ecuadorian Family Members						Unrelated Ecuadorian Controls ($n = 22$)	
			Affected ($n = 9$)		Unaffected ($n = 9$)		Unknown ($n = 3$)	All ($n = 21$)		Affected		Unaffected		All			
rsID	Chromosome Start Position, bp*	Polymorphism	*n*	%	*n*	%	*n*	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
(a) Segregated sequence variants																	
IL1RN (NM_173,841.2)																	
rs2071459	113,887,483	c.214+242C > T	9	100.00	3	33.33	0	12	57.14	6 ($n = 30$)	20.00	13 ($n = 60$)	21.67	19 ($n = 90$)	15.83	10	45.45
SLC4A11 (NM_001,174,089.1)																	
rs2281575	3,209,371	c.2193-18C > T	8	88.89	3	33.33	1	12	57.14	17 ($n = 29$)	58.62	36 ($n = 58$)	62.07	53 ($n = 87$)	60.92	11	50.00
-	3,208,726	c.2558+149_2558+203del54	8	88.89	2	22.22	1	11	52.38	0 ($n = 31$)	0.00	0 ($n = 58$)	0.00	0 ($n = 89$)	0.00	1	4.55
(b) Other sequence variants																	
IL1A (NM_000,575.3)																	
-	113,542,993	c.-982G > A	1	11.11	0	0.00	2	3	14.29								
rs1800587	113,542,960	c.-948C > T	7	77.78	7	77.78	0	14	66.67								
rs1609682	113,540,205	c.96+88C > A	8	88.89	9	100.00	1	18	85.71								
rs1894399	113,540,177	c.96+116G > A	7	77.78	7	77.78	1	15	71.43								
rs2071374	113,537,352	c.320-109A > C	2	22.22	6	66.67	0	8	38.10								
rs2856841	113,537,339	c.320-96T > C	6	66.67	7	77.78	1	14	66.67								
rs17561	113,537,223	c.340G > T	7	77.78	6	66.67	1	14	66.67								
rs3783550	113,532,885	c.616-41C > A	8	88.89	9	100.00	1	18	85.71								
rs1304037	113,532,236	c.1224A > G	7	78.00	8	89.00	1	16	76.19								
rs16347	113,531,719	c.*924_*925insTTCA	7	77.78	4	44.44	1	12	57.14								
IL1B (NM_000,576.2)																	
rs16944	113,594,867	c.-598T > C	9	100.00	8	88.89	2	19	90.48								
rs1143627	113,594,387	c.-118C > T	9	100.00	9	100.00	2	20	95.24								
-	113,593,089	c.99+54G > T	0	0.00	0	0.00	1	1	4.76								
rs3917359	113,591,162	c.100-10C > T	1	11.11	0	0.00	3	4	19.05								
rs1143633	113,590,467	c.302-64G > A	7	77.78	9	100.00	1	17	80.95								
rs1143634	113,590,390	c.315C > T	9	100.00	5	55.56	1	15	71.43								
rs1143639	113,588,793	c.597+76G > A	9	100.00	4	44.44	0	13	61.90								
rs34159283	113,588,758	c.597+111delTT	9	100.00	4	44.44	0	13	61.90								
rs1143643	113,588,302	c.598-152G > A	7	77.78	9	100.00	1	17	80.95								
rs1071676	113,587,433	c.1315G > C	9	100.00	5	56.00	0	14	66.67								
IL1RN (NM_173,841.2)																	
rs2234676	113,875,428	c.-168G > A	4	44.44	6	66.67	3	13	62.00								
rs2234677	113,875,509	c.-86G > A	4	44.44	6	66.67	3	13	62.00								
rs2234678	113,875,565	c.-30A > G	4	44.44	6	66.67	3	13	62.00								
rs2234679	113,875,584	c.-11G > C	4	44.44	6	66.67	3	13	62.00								
rs16065	113,875,631	c.10+26T > C	4	44.44	6	66.67	3	13	62.00								
rs4251969	113,875,753	c.10+148C > G	4	44.44	6	66.67	3	13	62.00								
rs4251970	113,875,773	c.10+168A > G	4	44.44	6	66.67	3	13	62.00								
rs4251986	113,877,538	c.11-105G > C	4	44.44	6	66.67	3	13	62.00								

Table 2. Continued

rsID	Chromosome Start Position, bp*	Polymorphism	KTCN-019 Affected (*n* = 9) *n*	%	KTCN-019 Unaffected (*n* = 9) *n*	%	KTCN-019 Unknown (*n* = 3) *n*	KTCN-019 All (*n* = 21) *n*	%	Other Ecuadorian Family Members Affected *n*	%	Other Ecuadorian Family Members Unaffected *n*	%	Other Ecuadorian Family Members All *n*	%	Unrelated Ecuadorian Controls (*n* = 22) *n*	%
rs878972	113,877,713	c.73+8A > C	4	44.44	4	44.44	3	11	52.00								
rs56079611	113,877,730	c.73+25T > G	4	44.44	5	55.56	0	9	43.00								
rs419598	113,887,207	c.180T > C	4	44.44	6	66.67	3	13	62.00								
rs423904	113,887,262	c.214+21C > T	4	44.44	6	66.67	3	13	62.00								
rs446433	113,887,273	c.214+32G > A	4	44.44	6	66.67	3	13	62.00								
rs495282	113,887,294	c.214+53G > C	4	44.44	6	66.67	3	13	62.00								
rs2232354	113,887,335	c.214+94T > G	4	44.44	5	55.56	0	9	43.00								
rs495410	113,887,338	c.214+97A > C	4	44.44	6	66.67	3	13	62.00								
rs442710	113,887,399	c.214+158G > A	4	44.44	6	66.67	3	13	62.00								
rs408392	113,887,458	c.214+217G > T	4	44.44	6	66.67	3	13	62.00								
rs451578	113,888,557	c.215-65G > A	4	44.44	6	66.67	3	13	62.00								
rs432014	113,888,579	c.215-43T > C	4	44.44	6	66.67	3	13	62.00								
rs454078	113,888,793	c.327+59A > T	4	44.44	6	66.67	3	13	62.00								
rs315952	113,890,304	c.399T > C	9	100.00	6	66.67	1	16	76.00								
rs315951	113,890,586	c.*138C > G	9	100.00	6	66.67	1	16	76.00								
-	113,890,928	c.*480_481delT	9	100.00	9	100.00	3	21	100.00								
rs9005	113,891,412	c.*964G > A	4	44.00	5	56.00	2	11	52.00								
SLC4A11 (NM_001,174,089.1)																	
rs6107260	3,219,894	c.43-58T > C	8	88.89	6	66.67	3	17	80.95								
rs6084314	3,219,692	c.43+49G > A	0	0.00	1	11.11	2	3	14.29								
rs113139885	3,219,561	c.43+180C > T	9	100.00	4	44.44	1	14	66.67								
rs3827076	3,218,355	c.44-121G > C	0	0.00	1	11.11	2	3	14.29								
rs6037508	3,217,989	c.88+201A > C	9	100.00	5	55.56	1	15	71.43								
rs3842433	3,215,077	c.292-118_292-117insGGGCTGG	0	0.00	1	11.11	2	3	14.29								
rs6139040	3,215,046	c.292-86G > C	9	100.00	5	55.56	1	15	71.43								
rs3803956	3,214,581	c.591G > A	8	88.89	4	44.44	1	13	61.90								
-	3,214,214	c.641G > A	7	77.78	7	77.78	3	17	80.95								
rs3803955	3,214,126	c.729+34G > A	0	0.00	2	22.22	0	2	9.52								
rs2144771	3,214,020	c.729+140C > A	9	100.00	7	77.78	2	18	85.71								
rs74174550	3,212,223	c.730-31_730-29delTCTinsCCAC	9	100.00	5	55.56	0	14	66.67								
rs41281862	3,211,304	c.1283-11C > T	0	0.00	2	22.22	0	2	9.52								
rs6084312	3,211,235	c.1341G > A	0	0.00	2	22.22	0	2	9.52								
rs3810561	3,211,064	c.1415+97T > G	9	100.00	9	100.00	3	21	100.00								
rs41281858	3,209,083	c.2389-9C > T	0	0.00	3	33.33	0	3	14.29								
rs139017795	3,208,741	c.2558+164G > A	0	0.00	1	11.11	0	1	4.76								
rs148132505	3,208,406	c.*27C > T	0	0.00	1	11.00	0	1	5.00								

* NCBI build 37.1 genome assembly.

117insGGGCTGG, c.730-31_730-29delTCTinsCCAC, and c.2558+149_2558+203del54. Among detected variants, both c.641G > A and c.2558+149_2558+203del54 were novel. The c.641G > A substitution is located in the coding sequence of *SLC4A11* gene. However, it does not cause the change in the protein sequence (Gly241Gly). Another two variants located in coding sequence (c.591G > A and c.1341G > A) were observed. They also do not change the amino acid residue in the protein encoded by the *SLC4A11* gene (Ser197Ser and Thr447Thr, respectively).

In the *SLC4A11* gene, the polymorphism c.2193-18C > T was observed in all but one affected individual, three healthy family members, and one person of unknown phenotype. This sequence variant was observed in 11 healthy individuals among the 22 Ecuadorian individuals and in 53 individuals from other KTCN families (17 affected and 36 unaffected).

The deletion of 54 nucleotides located in intron 19 of the *SLC4A11* gene was observed in all but one affected individual, two healthy individuals, and one person of unknown phenotype ($P = 0.00761$). All deletion carriers were heterozygous for the deletion. In addition, 9 of 10 deletion carriers in the KTCN-019 family had the same haplotype (4 – 10 – 15) for markers D20S906, D20S842, and D20S889, marked in blue in the Figure. This sequence variant was observed in one healthy person among the 22 Ecuadorian individuals and was not identified in other KTCN families.

Polyphen-2/SIFT Analysis

Analysis performed with the use of PolyPhen-2 for the Ala114Ser substitution in IL1-α pointed to a "possibly damaging" status with a score of 0.881 (sensitivity: 0.82; specificity: 0.94) for the HumDiv-trained model, whereas the SIFT analysis defined the substitution as tolerated for the IL1-α structure and function (score of 0.51).

Human Splicing Finder Analysis

Difference in splice pattern between wild sequence of *IL1RN* gene and the c.214+242C > T substitution was analyzed. In sequence with polymorphism, according to HSF Matrices and MaxEnt, new splice sites were not identified. The highest CV differences were observed for two silencer motifs. Variation for wild sequence of motif TCTCCCAA was estimated at 79.82, and 62.64 for sequence with c.214+242C > T (ΔCV: −21.53%). For motif [T/G]G[T/A]GGGG, variation for the wild sequence was 60.31 and 70.18 for polymorphism sequence (ΔCV: +16.37%).

Analysis of sequence variant c.2558+149_2558+203del54 in *SLC4A11* gene revealed that a new acceptor site is created in the deletion junction. Five base pairs (cctag) of the sequence motif cctag_ggtggagAG originate from the 5′ sequence fragment, whereas the following nine base pairs belong to the retained unchanged sequence fragment. With the use of HSF Matrices, CV = 11.79 and CV = 70.39 were predicted for the wild-type sequence and the sequence with deletion, respectively. The ΔCV was estimated at +496.95%.

Discussion

KTCN is considered as a multifactorial disorder, in which both environmental and genetic factors are involved. The identification of numerous KTCN loci in different populations indicates polygenic bases of the disease. Hughes et al.[49] detected the mutation in the seed region of *miR-184*, located at the 15q22-q25 locus. This chromosomal region was previously recognized as the KTCN locus for a Northern Irish family.[50] Furthermore, mutation in *DOCK9*, localized at the KTCN 13q32 locus, was observed.[51] Additionally both *VSX1* and *SOD1* genes are widely analyzed in KTCN etiology. Although some disease-causing mutations in *VSX1* and *SOD1* genes have been identified, subsequent studies have not confirmed these results.[11–20] Both *VSX1* and *SOD1* were analyzed in Ecuadorian families with KTCN, including the KTCN-019 family; however, these genes have been excluded as KTCN-causing in studied families, as described in a previous study.[15]

In this study, nonparametric linkage analysis carried out for members of an Ecuadorian family has indicated two novel suggestive KTCN loci, at 2q13-q14.3 and 20p13-p12.2. Those chromosomal regions have not been discussed earlier in the context of familiar form of KTCN. Reconstruction of haplotype for the 2q13-q14.3 chromosomal region has not revealed full segregation with KTCN phenotype in the studied family and that indicates an autosomal dominant inheritance mode with reduced penetrance. Difficulties in obtaining a clear pattern of inheritance for the KTCN-019 family might confirm the multifactorial basis of KTCN.

Three candidate genes, *IL1A*, *IL1B*, and *IL1RN*, at locus 2q13-q14.3 were selected for further analyses. These genes encode proteins belonging to the IL-1 superfamily. IL-1 is a pleiotropic cytokine that is involved in stimulating immune responses, such as inflammation, and regulates cell growth, differentiation, and motility of cells. It is secreted in response to various antigens of viral, bacterial, and fungal infections. IL-1 influences the adaptive immune system processes, affecting the development of B-cells, inducing secretion of IL-6 by T cells and stimulates the release of other proinflammatory cytokines: IFN-γ or tumor necrosis factor TNF.

Genes coding proteins of the IL-1 family are expressed in many cell types, including keratocytes.[31,52–58] Wilson et al.[31] proposed that contribution of proteins from the IL-1 family in apoptosis of cornea's cells might cause the development of KTCN. Their studies showed a relationship between the concentration of IL-1 proteins in the cell, and the way they affect the processes occurring in the cell. Observations presented by Wilson et al.[31] indicated that high concentrations of IL-1 caused programmed cell death, whereas lower concentrations stimulated the negative chemotaxis of cells.

A possible involvement of IL-1 in the development of KTCN appears to correlate with the Macé et al.[59] hypothesis that in corneas affected with KTCN, abnormal apoptotic signaling pathways affect keratocyte proliferation in response to minor damage of the cornea, and consequently affect cell density in the cornea.

Chosen polymorphisms of *IL1A*, *IL1B*, and *IL1RN* genes were tested in a case-control study in a Korean population.[8] Analyzed genes were selected based on the research study, which demonstrated that keratocyte apoptosis observed in 60% of KTCN corneas was triggered by the epithelial release of IL-1 as a consequence of chronic mechanical injury to the corneal epithelium.[37] The authors indicated a statistically significant association of two polymorphisms: rs16944 and rs1143627 with KTCN. Both variants are located in the promoter of the *IL1B* gene, and have had an impact on the concentration of IL-1α and IL-1β in the cells.[60,61] Our studies have led to the identification of these polymorphisms in both affected an unaffected family members, suggesting no relationship between rs16944 and rs1143627 and KTCN in the studied Ecuadorian family. Furthermore, sequence analysis of *IL1B* indicated a random distribution of other sequence variants identified in the KTCN-019 family confirming lack of role of this gene in the studied family.

The genetic screening of *IL1A* has revealed only one nonsynonymous substitution Ala114Ser at c.340G > T. Poly-

Phen-2 pointed to the Ala114Ser substitution as "possibly damaging," whereas SIFT assessed the impact of Ala114Ser substitution as tolerated for structure and function of IL-1α. The substitution Ala114Ser is very frequent in other populations. For example, allele T at position c.340 was observed in almost 30% of European and 25% of American individuals from the 1000 Genomes Project, phase 1. The c.340G > T substitution is present in both affected and unaffected Ecuadorian family members, suggesting that has no influence on the KTCN phenotype in the studied family.

In this study, the sequence of a gene that encodes IL-1Ra was also examined. The IL-1Ra binds to IL-1 type I receptors (IL-1RIs), preventing its association with the IL-1 receptor accessory protein (IL-1RAcP). This inhibits the interaction between IL-1 and its cell surface receptors, which competitively inhibits the effects of IL-1.[62] IL-1Ra exists in four different isoforms, among which one isoform (sIL-1Ra) is secreted, and the three others (icIL-1Ra1, icIL-1Ra2, and icIL-1Ra3) are intracellular.[63] The sIL-1Ra and icIL-1Ra1 mRNAs are generated from different promoters and contain isoform-specific 5′ sequences due to alternative splicing.[64,65] Whereas mRNAs for both icIL-1Ra1 and icIL-1Ra2 are transcribed using the same promoter, the following alternative splicing for icIL-1Ra2 results in additional amino acids.[66] The icIL-1Ra3 is a shortened variant of sIL-1Ra, created either by alternative translational initiation or alternative splicing.[67] Sequence variant c.214+242C > T, mapped in the fourth intron of the *IL1RN* gene, was observed in all affected members of the family KTCN-019 and in three healthy family members. For all c.214+242C > T substitution carriers, the haplotype 2 – 1 for the microsatellite markers D2S160 and D2S347 was also observed. The results indicated a suggestive link between locus 2q13-q14.3 and KTCN in the KTCN-019 family. However, random distribution of this sequence variant in affected and unaffected individuals in both other KTCN families and Ecuadorian healthy individuals suggests the influence of *IL1RN* gene on KTCN in this particular family only.

The linkage analysis in the family KTCN-019 also indicated locus 20p13-p12.2 in which candidate gene *SLC4A11* was selected. The protein encoded by the *SLC4A11* gene is involved in the transport of borate ions. However, in the absence of borate, SLC4A11 acts as an Na^+ and OH^- (H^+) channel. This transmembrane protein is involved in growth and cell proliferation, by commissioning of a mitogen-activated kinase pathway.[68,69] The *SLC4A11* gene is highly expressed in blood, ovary, tongue, lungs, skin, colon, and corneal endothelial cells.[70,71] There are many mutations described in *SLC4A11*-related corneal diseases, including recessive corneal endothelial dystrophy 2, corneal dystrophy and perceptive deafness, and Fuchs endothelial corneal dystrophy.[71–74] However, *SLC4A11* mutations have never been described to be associated with KTCN.

Recent study indicated that failure in SLC4A11 function leads to apoptosis[36] and it is known that apoptosis can be related to KTCN.[37] In light of these results, we recognized *SLC4A11* gene as a good KTCN candidate. In our study, among 20 different sequence variants of *SLC4A11* identified in family KTCN-019, the deletion of 54 nucleotides at position c.2558+149_2558+203 was observed in eight patients, two healthy individuals, and one person of unknown phenotype. Reconstruction of haplotype for microsatellite markers D20S906, D20S842, and D20S889 revealed haplotype 4 – 10 – 15 which was shared by nine deletion carriers, suggesting the autosomal dominant model of inheritance. In the in silico analysis with the use of HSF Matrices, a new acceptor site was predicted. This motif was created as a result of the junction of the deletion breakpoint sequences. The deletion was not observed in other Ecuadorian families; however, further analyses are necessary to evaluate the role of the c.2558+149_2558+203del54 in gene splicing.

Summarizing, in this study, two suggestive loci correlated with KTCN were identified. Next, during evaluation of candidate genes from these loci, several sequence variants were recognized, including five novel variants. The c.2558+149_2558+203del54 in *SLC4A11* and c.214+242C > T in *IL1RN* sequence variants may play a role in KTCN etiology in the examined Ecuadorian family. However, because obtained results are specific to the KTCN-019 family, only further studies of these sequence variants are necessary to be performed in other populations.

References

1. Rabinowitz M. Keratoconus. *Surv Ophthalmol*. 1998;42:297–319.
2. Nowak DM, Gajecka M. The genetics of keratoconus. *Middle East Afr J Ophthalmol*. 2011;18:2–6.
3. Wang Y, Rabinowitz YS, Rotter JI, Yang H. Genetic epidemiological study of keratoconus: evidence for major gene determination. *Am J Med Genet*. 2000;93:403–409.
4. Edwards M, McGhee CN, Dean S. The genetics of keratoconus. *Clin Experiment Ophthalmol*. 2001;29:345–351.
5. Levy D, Hutchings H, Rouland JF, et al. Videokeratographic anomalies in familial keratoconus. *Ophthalmology*. 2004;111:867–874.
6. Tang YG, Rabinowitz YS, Taylor KD, et al. Genomewide linkage scan in a multigeneration Caucasian pedigree identifies a novel locus for keratoconus on chromosome 5q14.3-q21.1. *Genet Med*. 2005;7:397–405.
7. Bisceglia L, De Bonis P, Pizzicoli C, et al. Linkage analysis in keratoconus: replication of locus 5q21.2 and identification of other suggestive loci. *Invest Ophthalmol Vis Sci*. 2009;50:1081–1086.
8. Kim S-H, Mok J-W, Kim H-S, Joo CK. Association of –31T>C and –511 C>T polymorphisms in the interleukin 1 beta (IL1B) promoter in Korean keratoconus patients. *Mol Vis*. 2008;14:2109–2116.
9. Burdon KP, Macgregor S, Bykhovskaya Y, et al. Association of polymorphisms in the hepatocyte growth factor gene promoter with keratoconus. *Invest Ophthalmol Vis Sci*. 2011;52:8514–8519.
10. Li X, Bykhovskaya Y, Haritunians T, et al. A genome-wide association study identifies a potential novel gene locus for keratoconus, one of the commonest causes for corneal transplantation in developed countries. *Hum Mol Genet*. 2012;21:421–429.
11. Héon E, Greenberg A, Kopp KK, et al. VSX1: a gene for posterior polymorphous dystrophy and keratoconus. *Hum Mol Genet*. 2002;11:1029–1036.
12. Eran P, Almogit A, David Z, et al. The D144E substitution in the VSX1 gene: a non-pathogenic variant or a disease causing mutation? *Ophthalmic Genet*. 2008;29:53–59.
13. Bisceglia L, Ciaschetti M, De Bonis P, et al. VSX1 mutational analysis in a series of Italian patients affected by keratoconus: detection of a novel mutation. *Invest Ophthalmol Vis Sci*. 2005;46:39–45.
14. Aldave AJ, Yellore VS, Salem AK, et al. No VSX1 gene mutations associated with keratoconus. *Invest Ophthalmol Vis Sci*. 2006;47:2820–2822.
15. Gajecka M, Radhakrishna U, Winters D, et al. Localization of a gene for keratoconus to a 5.6-Mb interval on 13q32. *Invest Ophthalmol Vis Sci*. 2009;50:1531–1539.

16. Tang YG, Picornell Y, Su X, Li X, Yang H, Rabinowitz YS. Three VSX1 gene mutations, L159M, R166W, and H244R, are not associated with keratoconus. *Cornea*. 2008;27:189–192.
17. Liskova P, Ebenezer ND, Hysi PG, et al. Molecular analysis of the VSX1 gene in familial keratoconus. *Mol Vis*. 2007;13:1887–1891.
18. Udar N, Atilano SR, Brown DJ, et al. SOD1: a candidate gene for keratoconus. *Invest Ophthalmol Vis Sci*. 2006;47:3345–3351.
19. Stabuc-Silih M, Strazisar M, Hawlina M, Glavac D. Absence of pathogenic mutations in VSX1 and SOD1 genes in patients with keratoconus. *Cornea*. 2010;29:172–176.
20. Saee-Rad S, Hashemi H, Miraftab M, et al. Mutation analysis of VSX1 and SOD1 in Iranian patients with keratoconus. *Mol Vis*. 2011;17:3128–3136.
21. Jacq PL, Sale Y, Cochener B, Lozach P, Colin J. Keratoconus changes in corneal topography and allergy. Study of 3 groups of patients. *J Fr Ophtalmol*. 1997;20:97–102.
22. Gasset AR, Houde WL, Garcia-Bengochea M. Hard contact lens wear as an environmental risk in keratoconus. *Am J Ophthalmol*. 1978;85:339–341.
23. Rahi A, Davies P, Ruben M, Lobascher D, Menon J. Keratoconus and coexisting atopic disease. *Br J Ophthalmol*. 1977;61:761–764.
24. Wachtmeister L, Ingemansson SO, Möller E. Atopy and HLA antigens in patients with keratoconus. *Acta Ophthalmol (Copenh)*. 1982;60:113–122.
25. Harrison RJ, Klouda PT, Easty DL, Manku M, Charles J, Stewart CM. Association between keratoconus and atopy. *Br J Ophthalmol*. 1989;73:816–822.
26. Gondhowiardjo TD, Van Haeringen NJ. Corneal aldehyde dehydrogenase, glutathione reductase, and glutathione S-transferase in pathologic corneas. *Cornea*. 1993;12:310–314.
27. Gondhowiardjo TD, Van Haeringen NJ, Völker-Dieben HJ, et al. Analysis of corneal aldehyde dehydrogenase patterns in pathologic corneas. *Cornea*. 1993;12:146–154.
28. Kenney MC, Nesburn AB, Burgeson RE, Butkowski RJ, Ljubimov AV. Abnormalities of the extracellular matrix in keratoconus corneas. *Cornea*. 1997;16:345–351.
29. Kolozsvári L, Nógrádi A, Hopp B, Bor ZUV. Absorbance of the human cornea in the 240- to 400-nm range. *Invest Ophthalmol Vis Sci*. 2002;43:2165–2168.
30. Balasubramanian SA, Pye DC, Willcox MDP. Are proteinases the reason for keratoconus? *Curr Eye Res*. 2010;35:185–191.
31. Wilson SE, He Y-G, Weng J, et al. Epithelial injury induces keratocyte apoptosis: hypothesized role for the interleukin-1 system in the modulation of corneal tissue organization and wound healing. *Exp Eye Res*. 1996;62:325–338.
32. Sims J, March C, Cosman D, et al. cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. *Science*. 1988;241:585–589.
33. Steinkasserer A, Spurr NK, Cox S, Jeggo P, Sim RB. The human IL-1 receptor antagonist gene (IL1RN) maps to chromosome 2q14-q21, in the region of the IL-1 alpha and IL-1 beta loci. *Genomics*. 1992;13:654–657.
34. Romero MF, Fulton CM, Boron WF. The SLC4 family of HCO3 - transporters. *Pflugers Arch*. 2004;447:495–509.
35. Cordat E, Casey JR. Bicarbonate transport in cell physiology and disease. *Biochem J*. 2009;417:423.
36. Liu J, Seet L-F, Koh LW, et al. Depletion of SLC4A11 causes cell death by apoptosis in an immortalized human corneal endothelial cell line. *Invest Ophthalmol Vis Sci*. 2012;53:3270–3279.
37. Kim WJ, Rabinowitz YS, Meisler DM, Wilson SE. Keratocyte apoptosis associated with keratoconus. *Exp Eye Res*. 1999;69:475–481.
38. Wigginton JE, Abecasis GR. PEDSTATS: descriptive statistics, graphics and quality assessment for gene mapping data. *Bioinformatics*. 2005;21:3445–3447.
39. Sobel E, Lange K. Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker-sharing statistics. *Am J Hum Genet*. 1996;58:1323–1337.
40. Lange EM, Lange K. Powerful allele sharing statistics for nonparametric linkage analysis. *Hum Hered*. 2004;57:49–58.
41. Mukhopadhyay N, Almasy L, Schroeder M, Mulvihill WP, Weeks DE. Mega2: data-handling for facilitating genetic linkage and association analyses. *Bioinformatics*. 2005;21:2556–2557.
42. Matise TC, Chen F, Chen W, et al. A second-generation combined linkage–physical map of the human genome. *Genome Res*. 2007;17:1783–1786.
43. Weeks DE, Sobel E, O'Connell JR, Lange K. Computer programs for multilocus haplotyping of general pedigrees. *Am J Hum Genet*. 1995;56:1506–1507.
44. Thiele H, Nürnberg P. HaploPainter: a tool for drawing pedigrees with complex haplotypes. *Bioinformatics*. 2005;21:1730–1732.
45. Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol*. 2000;132:365–386.
46. Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. *Nucleic Acids Res*. 2002;30:3894–3900.
47. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Prot*. 2009;4:1073–1081.
48. Desmet F-O, Hamroun D, Lalande M, Collod-Béroud G, Claustres M, Béroud C. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. *Nucleic Acids Res*. 2009;37:e67.
49. Hughes AE, Bradley DT, Campbell M, et al. Mutation altering the miR-184 seed region causes familial keratoconus with cataract. *Am J Hum Genet*. 2011;89:628–633.
50. Hughes AE, Dash DP, Jackson AJ, Frazer DG, Silvestri G. Familial keratoconus with cataract: linkage to the long arm of chromosome 15 and exclusion of candidate genes. *Invest Ophthalmol Vis Sci*. 2003;44:5063–5066.
51. Czugala M, Karolak JA, Nowak DM, et al. Novel mutation and three other sequence variants segregating with phenotype at keratoconus 13q32 susceptibility locus. *Eur J Hum Genet*. 2012;20:389–397.
52. Boxman I, Löwik C, Aarden L, Ponec M. Modulation of IL-6 production and IL-1 activity by keratinocyte-fibroblast interaction. *J Invest Dermatol*. 1993;101:316–324.
53. Das S, Potter H. Expression of the Alzheimer amyloid-promoting factor antichymotrypsin is induced in human astrocytes by IL-1. *Neuron*. 1995;14:447–456.
54. Ishikura H, Takahashi C, Kanagawa K, Hirata H, Imai K, Yoshiki T. Cytokine regulation of ICAM-1 expression on human renal tubular epithelial cells in vitro. *Transplantation*. 1991;51:1272–1275.
55. Kavita U, Mizel SB. Differential sensitivity of interleukin-1 alpha and -beta precursor proteins to cleavage by calpain, a calcium-dependent protease. *J Biol Chem*. 1995;270:27758–27765.
56. Kern JA, Lamb RJ, Reed JC, Elias JA, Daniele RP. Interleukin-1-beta gene expression in human monocytes and alveolar macrophages from normal subjects and patients with sarcoidosis. *Am Rev Respir Dis*. 1988;137:1180–1184.
57. Matsunaga T, Katayama I, Yokozeki H, Nishioka K. Superantigen-induced cytokine expression in organ-cultured human skin. *J Dermatol Sci*. 1996;11:104–110.
58. Zola H, Flego L, Wong YT, Macardle PJ, Kenney JS. Direct demonstration of membrane IL-1 alpha on the surface of

circulating B lymphocytes and monocytes. *J Immunol*. 1993; 150:1755–1762.

59. Macé M, Galiacy SD, Erraud A, et al. Comparative transcriptome and network biology analyses demonstrate antiproliferative and hyperapoptotic phenotypes in human keratoconus corneas. *Invest Ophthalmol Vis Sci*. 2011;52: 6181–6191.
60. Chang Y-W, Jang J-Y, Kim N-H, et al. Interleukin-1B (IL-1B) polymorphisms and gastric mucosal levels of IL-1beta cytokine in Korean patients with gastric cancer. *Int J Cancer*. 2005;114: 465–471.
61. Chen H, Wilkins LM, Aziz N, et al. Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. *Hum Mol Genet*. 2006;15:519–529.
62. O'Neill LA, Dinarello CA. The IL-1 receptor/toll-like receptor superfamily: crucial receptors for inflammation and host defense. *Immunol Today*. 2000;21:206–209.
63. Arend WP, Malyak M, Guthridge CJ, Gabay C. Interleukin-1 receptor antagonist: role in biology. *Annu Rev Immunol*. 1998;16:27–55.
64. Hannum CH, Wilcox CJ, Arend WP, et al. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature*. 1990;343:336–340.
65. Haskill S, Martin G, Van Le L, et al. cDNA cloning of an intracellular form of the human interleukin 1 receptor antagonist associated with epithelium. *Proc Natl Acad Sci U S A*. 1991;88:3681–3685.
66. Muzio M, Polentarutti N, Sironi M, et al. Cloning and characterization of a new isoform of the interleukin 1 receptor antagonist. *J Exp Med*. 1995;182:623–628.
67. Malyak M, Guthridge JM, Hance KR, Dower SK, Freed JH, Arend WP. Characterization of a low molecular weight isoform of IL-1 receptor antagonist. *J Immunol*. 1998;161:1997–2003.
68. Park M, Li Q, Shcheynikov N, Zeng W, Muallem S. NaBC1 is a ubiquitous electrogenic Na+-coupled borate transporter essential for cellular boron homeostasis and cell growth and proliferation. *Mol Cell*. 2004;16:331–341.
69. Park M, Li Q. Borate transport and cell growth and proliferation: not only in plants. *Cell Cycle*. 2005;4:24–26.
70. Parker MD, Ourmozdi EP, Tanner MJA. Human BTR1, a new bicarbonate transporter superfamily member and human AE4 from kidney. *Biochem Biophys Res Commun*. 2001;282: 1103–1109.
71. Vithana EN, Morgan P, Sundaresan P, et al. Mutations in sodium-borate cotransporter SLC4A11 cause recessive congenital hereditary endothelial dystrophy (CHED2). *Nat Genet*. 2006;38:755–757.
72. Desir J, Moya G, Reish O, et al. Borate transporter SLC4A11 mutations cause both Harboyan syndrome and non-syndromic corneal endothelial dystrophy. *J Med Genet*. 2007;44:322–326.
73. Jiao X, Sultana A, Garg P, et al. Autosomal recessive corneal endothelial dystrophy (CHED2) is associated with mutations in SLC4A11. *J Med Genet*. 2007;44:64–68.
74. Vithana EN, Morgan PE, Ramprasad V, et al. SLC4A11 mutations in Fuchs endothelial corneal dystrophy. *Hum Mol Genet*. 2008;17:656–666.