VSX1 gene analysis in keratoconus

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Purpose: To screen the visual system homeobox 1 (VSX1) gene in keratoconus patients.

Methods: The entire coding region of VSX1, including intron-exon boundaries were amplified in keratoconus cases (n=50) and controls (n=50). All sequences were analyzed against the ensemble sequence (ENSG00000100987) for VSX1.

Results: Sequencing analysis showed four alterations (p.A182A, p.R217H, p.P237P, and g.25059612C>T) in VSX1 of which g.25059612C>T (in intron 2) was found to be novel. Of these four, p.A182A and p.P237P were present in both cases as well as controls while p.R217H and g.25059612C>T were limited to cases only. All these changes were non-pathogenic.

Conclusions: In our study no pathogenic VSX1 mutation was identified. The role of VSX1 in the pathogenesis of keratoconus is still controversial. VSX1 mutations are responsible for a very small fraction of all observed keratoconus cases. The absence of pathogenic mutations in VSX1 in our patients indicates that other genetic loci like 13q32 as suggested by a recent study may be involved in the pathogenesis of this disorder.

Keratoconus (KTCN; OMIM 148300) is a bilateral, non-inflammatory, and progradient corneal ectasia [1]. There is no specific treatment for keratoconus except corneal transplantation. It has an estimated incidence between 1/500 to 1/2,000 persons throughout the world. The disease usually arises in the teens and stabilizes in the third and fourth decade of life [2]. It occurs with no ethnic or gender preponderance and causes significant visual impairment. Most cases of keratoconus are sporadic but some (5%–10%) have a positive family history [2,3]. In such cases both autosomal dominant and recessive patterns of inheritance have been documented [4-6]. The exact pathogenesis of keratoconus is still unknown. Genome-wide linkage analyses has identified several chromosomal loci and genes that may be associated with keratoconus [6-9]; however, some were eventually excluded [10,11] while for others a conclusive association with the disease is yet to be established. Mutations in the visual system homeobox 1 (VSX1) gene in keratoconus have been reported in different studies [12-15].

VSX1 is a member of the Vsx1 group of vertebrate paired-like homeodomain transcription factors. It has been localized to human chromosome 20p11-q11. Initially VSX1 was chosen for screening mutations in posterior polymorphous corneal dystrophy (PPCD) and keratoconus [8]. VSX1 is considered important in ocular development and is particularly involved in the developing cornea. Expression in human was demonstrated in embryonic craniofacial, adult corneal, and adult retinal cDNA libraries [16]. VSX1 mRNA has been found in the outer tier of the inner nuclear layer of the human retina and the cornea [17]. Mutations in this gene are also associated with posterior polymorphous dystrophy. VSX1 is highly conserved across many species [18-20]. Several mutations, such as p.D144E, p.G160D, p.P247R, p.L159M, p.R166W, and p.H244R have been reported by various groups [14,17,21] but a definite pathogenic role of these mutations in keratoconus is not yet established. In this study we present the results of VSX1 gene analysis in 50 keratoconus patients and controls from north India.

METHODS

Patient selection and DNA isolation: The research followed the tenets of the Declaration of Helsinki in the treatment of the subject reported herein. The study was approved by institutional review board (IRB # IRB00006862) of the All India Institute of Medical Sciences (AIIMS) and all participants gave their written informed consent. A total of fifty keratoconus patients (Table 1) presented (during April 2009 to April 2010) at the Dr. R. P. Centre for Ophthalmic Sciences (AIIMS, New Delhi, India) were enrolled in this study. Clinical evaluation involved Ultrasonic Pachymetry,
| Patient ID | Age in years | Sex | Visual acuity in Snellen’s chart | Munsen sign | Vogt’s striae | Hydrops | Scarring | Keratometry in VKG (in diopters) | Ultrasonic pachymetry (in μm) | Key |
|------------|--------------|-----|----------------------------------|-------------|--------------|--------|---------|--------------------------------|------------------------------|-----|
| KC1        | 20           | F   | 6/12, 6/12                       | +           | -            | -      | -       | 45.62                                        | 56                           | M   |
| KC2        | 12           | M   | 6/12, 6/12                       | +           | +            | +      | -       | 52                                           | 49                           | F   |
| KC3        | 12           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 52                                           | 49                           | M   |
| KC4        | 20           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                          | 68                           | F   |
| KC5        | 20           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | M   |
| KC6        | 19           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC7        | 18           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | M   |
| KC8        | 14           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC9        | 20           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | M   |
| KC10       | 22           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC11       | 19           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | M   |
| KC12       | 17           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC13       | 20           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC14       | 18           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC15       | 22           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC16       | 18           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC17       | 22           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC18       | 15           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC19       | 20           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC20       | 20           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC21       | 18           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC22       | 16           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC23       | 22           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC24       | 21           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC25       | 18           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC26       | 17           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC27       | 20           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC28       | 18           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC29       | 20           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC30       | 11           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC31       | 19           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC32       | 20           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC33       | 17           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC34       | 16           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC35       | 20           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC36       | 17           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC37       | 10           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC38       | 25           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC39       | 21           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC40       | 18           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC41       | 26           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC42       | 22           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC43       | 14           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC44       | 23           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC45       | 16           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC46       | 18           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC47       | 22           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC48       | 14           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC49       | 18           | M   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |
| KC50       | 20           | F   | 6/12, 6/12                       | +           | +            | -      | -       | 45                                           | 68                           | F   |

Key: M=male; F=female; OD=right eye; OS=left eye; +=positive; -=negative; VKG=videokeratography

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videokeratography (VKG), Orbscan, visual testing, fundoscopy, slitlamp-biomicroscopy, and retinoscopy. Of these patients, 29 were males and 21 were females. The mean age of presentation was 18.2 years. Diagnosis of keratoconus involved the presence of characteristic topographic features, such as inferior or central corneal steepening, or an asymmetric bowtie pattern with skewing of the radial axes, and the presence of one or more of the following characteristic clinical features in one or both eyes: conical corneal deformation, munsen sign, corneal stromal thinning, a Fleischer ring or Vogt striae. All cases were sporadic without any family history.

All keratoconus cases secondary to causes like trauma, surgery, Ehlers Danlos syndrome, Osteogenesis Imperfecta, and pellucid marginal degeneration were excluded from the study.

After informed consent, detailed personal, medical and occupational history was collected and a family tree up to three generations was drawn. Fifty ethnically matched normal individuals without any ocular disorder were enrolled as controls. Health information was obtained from controls through the questionnaire; all underwent ophthalmological examination and blood sample (5 ml) was collected in EDTA (EDTA) vacutainers (Greiner Bio-One GmbH, Frickenhausen, Germany) from patients and controls for DNA extraction. DNA was extracted from whole blood samples of all patients and controls using the phenol-chloroform method. PCR and DNA sequencing: All the coding regions of VSX1 including exon-intron junctions were amplified using a set of five oligonucleotide primers (Table 2). Each reaction was performed in a 25 µl mixture containing 0.2 µM each primer, 0.5 U Taq DNA polymerase (Biogene, New Delhi, India), 2.5 µl 10× PCR buffer (Biogene) with 2.5 mM MgCl₂, and approximately 100 ng genomic DNA. Thermal cycling was performed in a thermal cycler (My Cycler; Biorad, Gurgaon, India) under the following conditions: initial denaturation for 3 min at 95 °C; 35 cycles of 94 °C for 30 s, 55-60 °C for 45 s, 72 °C for 60 s; and a final extension for 10 min at 72 °C.

All PCR products were analyzed on 1.8% agarose gel stained with ethidium-bromide (EtBr; 10 mg/ml). Agarose gels were analyzed using gel documentation system (Applied Biosystems, Carlsbad, CA). Amplified PCR products were purified using gel/PCR DNA fragments extraction kit (DF100; Geneaid Biotech Ltd., Sijhih City Taiwan). The purified PCR products were sent for sequencing at MCLAB (Molecular Cloning Laboratories, South San Francisco, CA). Nucleotide sequences were compared with the VSX1 ensembl reference sequence.

**Insilico analysis of missense mutations:** Two homology based programs PolyPhen (Polymorphism Phenotyping) and SIFT (Sorting Intolerant From Tolerant) analysis tool were used to predict the functional impact of missense changes identified in this study.

**PolyPhen** structurally analyzes an amino acid polymorphism and predicts whether that amino acid change is likely to be deleterious to protein function [22,23]. The prediction is based on the position-specific independent counts (PSIC) score derived from multiple sequence alignments of observations. PolyPhen scores of >2.0 indicate the polymorphism is probably damaging to protein function. Scores of 1.5–2.0 are possibly damaging, and scores of <1.5 are likely benign.

**SIFT** is a sequence homology-based tool that sorts intolerant from tolerant amino acid substitutions and predicts whether an amino acid substitution in a protein will have a phenotypic effect [24,25]. SIFT is based on the premise that protein evolution is correlated with protein function. Positions

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**TABLE 2. PRIMERS USED FOR VSX1 GENE AMPLIFICATION.**

| Exon | Forward primer (5'-3') | Reverse primer (5'-3') | Product size (bp) |
|------|------------------------|------------------------|------------------|
| 1    | CAGCTGATTGGAGCCCTTC    | CTCAGACCCTAGGGGACAGG   | 599              |
| 2    | GCACATAAAAATGTGGCTCA   | GCCTCTAGGAACCTGCAAGA   | 393              |
| 3    | CATTCAAGGTTGGGTTT    | TCTTTGTTGTCCCTTCAGCTA  | 419              |
| 4    | GATCCTGTGGGGGAGGAGAG  | CTGTCTTGGCTGTGGAAAT    | 394              |
| 5    | CCCCAGAGATGGCAGCTGAC  | TGGACAATTTTTTGCTTTTG   | 495              |

**TABLE 3. VSX1 SEQUENCE VARIANTS OBSERVED IN THIS STUDY.**

| Nucleotide Change | VSX1 transcript ID | Protein alteration | Exon/UTR/intron | Patients (n=50) | Controls (n=50) | Reference/SNP ID | Polyphen/SIFT prediction |
|-------------------|--------------------|-------------------|-----------------|----------------|----------------|-----------------|-------------------------|
| g.25059546A>G     | NM_014588          | p.A182A           | Exon 3          | 25/50          | 29/50          | [20]            | –                      |
| (rs12480307)      |                    |                   |                 |                |                |                 |                         |
| g.25059442G>A     | NM_0199425         | p.R217H           | Exon 3          | 1/50           | Absent         | [20]            | Novel                  |
| (rs1138482)       |                    |                   |                 |                |                |                 |                         |
| g.25059381T>A     | NM_0199425         | p.P237P           | Exon 3          | 18/50          | 14/50          | [20]            | –                      |
| (rs56157240)      |                    |                   |                 |                |                |                 |                         |
| g.25059612C>T     | –                  | –                 | Intron 2        | 3/50           | Absent         | Novel           | –                      |
important for function should be conserved in an alignment of the protein family, whereas unimportant positions should appear diverse in an alignment. Positions with normalized probabilities less than 0.05 are predicted to be deleterious and, those greater than or equal to 0.05 are predicted to be tolerated.

We have also used improved Splice Site predictor tool [26] for prediction the effect of an intronic nucleotide change on splicing.

RESULTS
DNA sequencing analysis of 50 patients and 50 controls revealed a total of four nucleotide changes (Table 3) of which one was novel and 3 have been previously reported. Details of these changes are given below.

Alanine182Alanine (p.A182A): In this mutation a single nucleotide adenine (A) was replaced by guanine (G) at g. 25059546 (rs12480307); c.546; codon 182 resulted in codon change GCA>GCG resulting in synonymous change p.ala182ala (p.A182A; Figure 1). This change was present as homozygous change in 25 cases and 29 controls.

Arginine217Histidine (p.R217H): In this mutation a single nucleotide thymine (T) was replaced by adenine at
position g.25059442 (rs6138482); cDNA position c.650; codon 217. This change resulted in a codon change from CGC>CAC resulting in non-synonymous change p.arg217his (p.R217H; Figure 2) in protein. This change was present in only one case and was homozygous but was absent in controls.

Proline237Proline (p.P237P): In this mutation a single nucleotide T was replaced by A at position g.25059381 (rs6157240); c.711; codon 237 resulted in a codon change CCT>CCA which predicts a synonymous change p.pro237pro (p.P237P; Figure 3). This change was present in 18/50 cases (7 were homozygous and 11 were heterozygous) being also present in 14/50 controls (9 were homozygous and 5 were heterozygous).

Cytosine to Thymine in intron 2: A novel single nucleotide change C>T at g.25059612 (Figure 4) was present in three cases but absent in controls. Alteration is located in 2nd intron (IVS3–24C>T). This change was registered at GenBank with accession number GU471016.

PolyPhen and SIFT analysis of p.R217H showed that it is non-pathogenic (SIFT score is >0.05 and PSIC score is <1.5).

Improved splice site predictor tool analysis of g.25059612C>T showed that this location (g.25059612) is not present at splice site and may not create splicing error in VSXI mRNA. Conclusively, no pathogenic change was identified in our patients.

**DISCUSSION**

In this study we analyzed VSXI in 50 unrelated keratoconus patients and controls from north India. In our patient’s, males were affected more than females. Mutations in VSXI gene have been identified in association with keratoconus [12,14,15,17,21]. Human VSXI is a member of the CVC domain containing paired-like class of homeoproteins. VSXI expression in humans was detected in embryonic craniofacial, adult retinal, and adult corneal tissues [17,27]. The role of VSXI in keratoconus is still ambiguous. Previous studies have shown that the pathogenesis of keratoconus is very complex and several genes and gene environmental interactions play a critical role in disease prognosis. In fact, VSXI may have a pleiotropic action among the tissues of the cornea leading to non-inflammatory corneal thinning disorders. Surv Ophthalmol 1986; 42:297-319. [PMID: 9493273]

In a recent study (2009) from India, VSXI was screened in 66 keratoconus cases and a potentially pathogenic change (p.Q175H) was identified in one case only [13]. In this study, the second from India, no pathogenic change was indentified in VSXI. Similar results have been published recently [29,32,33]. So lack of possibly pathogenic changes in VSXI gene in keratoconus patients suggests that mutations of VSXI could only be responsible for a very small fraction of all observed cases and need to be investigated in different populations. This also suggests that other genetic loci like 13q32 as suggested by Gajecka et al. [34] may be involved in the pathogenesis of keratoconus.

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**REFERENCES**

1. Krachmer JH, Feder RS, Belin MW. Keratoconus and related noninflammatory corneal thinning disorders. Surv Ophthalmol 1984; 28:293-322. [PMID: 6230745]
2. Rabinowitz YS. Keratoconus. Surv Ophthalmol 1998; 42:297-319. [PMID: 9493273]
3. Kennedy RH, Bourne WM, Dyer JA. A 48-year clinical and epidemiologic study of keratoconus. Am J Ophthalmol 1986; 101:267-73. [PMID: 3513592]
4. 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; 44:5063-6. [PMID: 14638698]
5. Wang Y, Rabinowitz YS, Rotter JI, Yang H. Genetic epidemiological study of keratoconus: evidence for major pathogenicity could not be confirmed as some of these variants were observed in unaffected individuals also. He/on and associates [14] identified a compound heterozygous change with p.P247R and p.G160D and reported p.G160D to be pathogenic and p.P247R to be nonpathogenic. Another study reported p.P247R as a pathogenic change because it was found to be co-segregating with keratoconus. Similarly, p.D144E mutation was initially reported as pathogenic [20,21] but subsequent studies identified its presence in unaffected individuals and suggested this to be a non-pathogenic polymorphism [29,30]. The variants p.R166W, p.H244R, and p.L159M have been identified in keratoconus patients but these changes did not segregate with the disease phenotype in their family members and hence were not considered sufficiently significant to support a pathogenic role in keratoconus [31]. Similarly, variants p.G160V and p.N151S have been identified in patients from the Korean population [12] but these changes have not been reported in other populations.

In this study, four sequence variations were detected of VSXI variants reported in various studies include p.L17P, p.D144E, p.N151S, p.L159M, p.G160V, p.G160D, p.R166W, p.Q175H, p.H244R, and p.P247R [12-15,17,21]. Some of these were initially reported to be pathogenic but their
12. Mok JW, Sistonen P, Tuupanen S, Tervo T, Dammert A, Latvala T, Altitalo T. A locus for autosomal dominant keratoconus: linkage to 16q22.3-q23.1 in Finnish families. Invest Ophthalmol Vis Sci 2002; 43:3160-4. [PMID: 12356819]

13. Paliwal P, Singh A, Tandon R, Titiyal JS, Sharma A. A novel mutation identified in an individual with keratoconus nonpathogenic variant or a disease causing mutation. Invest Ophthalmol Vis Sci 2005; 46:39-45. [PMID: 15623752]

14. Héon E, Greenberg A, Kopp KK, Rootman D, Vincent AL, Billingsley G, Priston M, Dorval KM, Chow RL, McInnes RR, Heathcote G, Westall C, Sutphin JE, Semina E, Bremner R, Stone EM. VSX1: a gene for posterior polymorphous dystrophy and keratoconus. Hum Mol Genet 2002; 11:1029-36. [PMID: 11978762]

15. Semina EV, Mintz-Hittner HA, Murray JC. Isolation and characterization of a novel human paired-like homeodomain-containing transcription factor gene, VSX1, expressed in ocular tissues. Genomics 2000; 63:289-93. [PMID: 10673340]

16. Hayashi T, Huang J, Deeb SS. RINX (VSX1), a novel homeobox gene expressed in the inner nuclear layer of the adult retina. Genomics 2000; 67:128-39. [PMID: 10903837]

17. Burmeister M, Novak J, Liang MY, Basu S, Plored L, Hawes NL, Vidgen D, Hoover F, Goldman D, Kalinski VI, Roderick TH, Taylor BA, Hankin MH, McInnes RR. Ocular retardation mouse caused by Chx10 homeobox null allele: impaired retinal progenitor proliferation and bipolar cell differentiation. Nat Genet 1996; 12:376-84. [PMID: 8630490]

18. Chow RL, Snow B, Novak J, Looser J, Freund C, Vidgen D, Plored L, McInnes RR. Vsx1, a rapidly evolving paired-like homeobox gene expressed in cone bipolar cells. Mech Dev 2001; 109:315-22. [PMID: 11731243]

19. Hughes AE, Dash DP, Jackson AJ, Frazer DG, Silvestri G. Northern Europe: a family with keratoconus caused by a novel mutation in the VSX1 gene. Invest Ophthalmol Vis Sci 2003; 44:5063-6. [PMID: 14638698]

20. Rabinowitz YS, Maumenee IH, Lundergan MK, Puffenberger O, Forrest S. Identity-by-descent approach to gene localization in autosomal dominant keratoconus maps to human chromosome 15 and exclusion of candidate genes. Invest Ophthalmol Vis Sci 2003; 44:5066-30. [PMID: 14638699]

21. Eran P, Almogit A, David Z, Wolf HR, Hana G, Yaniv B, Elon P, Isaac A. The D144E substitution in the VSX1 gene: a nonpathogenic variant or a disease causing mutation. Ophthalmic Genet 2008; 29:53-9. [PMID: 18484309]

22. Semina EV, Mintz-Hittner HA, Murray JC. Isolation and characterization of a novel human paired-like homeodomain-containing transcription factor gene, VSX1, expressed in ocular tissues. Genomics 2000; 63:289-93. [PMID: 10673340]

23. Stabuc-Silih M, Strazisar M, Hawlina M, Glavac D. Absence of pathogenic mutations in VSX1 and SOD genes in patients with keratoconus. Cornea 2008; 27:189-92. [PMID: 18216574]

24. Liskova P, Ebenezer ND, Hysi PG, Gwilliam R, El-Ashry MF, Moodaley LC, Hau S, Twu M, Tuft SJ, Bhattacharya SS. Molecular analysis of the VSX1 gene in familial keratoconus. Mol Vis 2006; 47:2820-2. [PMID: 16799019]

25. Korvatska E, Munier FL, Djemal A, Wang MX, Frueh B, Chiou AG, Uffer S, Ballestrazzi E, Braunein RE, Forster RK, Culbertson WW, Boman H, Zografos L, Schorderet DF. Mutation hot-spot in 5q31-linked corneal dystrophies. Am J Hum Genet 1998; 62:320-4. [PMID: 9463327]

26. Aldave AJ, Yellore VS, Salem AK, Yoo GL, Rayner SA, Yang H, Tang GY, Piconell Y, Rabinowitz YS. No VSX1 gene mutations associated with keratoconus. Invest Ophthalmol Vis Sci 2006; 47:2820-2. [PMID: 16799019]

27. Rozen S, Skaleitsky HJ. Bioinformatics Methods and Protocols: Methods in Molecular Biology. Totowa, NJ: Humana Press; 2000. p. 365–386.

28. Korvatska E, Munier FL, Djemal A, Wang MX, Frueh B, Chiou AG, Uffer S, Ballestrazzi E, Braunein RE, Forster RK, Culbertson WW, Boman H, Zografos L, Schorderet DF. Mutation hot-spot in 5q31-linked corneal dystrophies. Am J Hum Genet 1998; 62:320-4. [PMID: 9463327]

29. Liskova P, Ebenezer ND, Hysi PG, Gwilliam R, El-Ashry MF, Moodaley LC, Hau S, Twu M, Tuft SJ, Bhattacharya SS. Molecular analysis of the VSX1 gene in familial keratoconus. Mol Vis 2006; 47:2820-2. [PMID: 16799019]

30. Liskova P, Ebenezer ND, Hysi PG, Gwilliam R, El-Ashry MF, Moodaley LC, Hau S, Twu M, Tuft SJ, Bhattacharya SS. Molecular analysis of the VSX1 gene in familial keratoconus. Mol Vis 2006; 47:2820-2. [PMID: 16799019]
34. Gajecka M, Radhakrishna U, Winters D, Nath SK, Rydzanicz M, Ratnamala U, Ewing K, Molinari A, Pitarque JA, Lee K, Leal SM, Bejjani BA. Localization of a gene for keratoconus to a 5.6-Mb interval on 13q32. Invest Ophthalmol Vis Sci 2009; 50:1531-9. [PMID: 19011015]