ISSN 1022-7954, Russian Journal of Genetics, 2017, Vol. 53, No. 5, pp. 519–527. © Pleiades Publishing, Inc., 2017.
Original Russian Text © M.M. Bikbov, E.L. Usubov, K.Kh. Oganisyan, S.L. Lobov, R.R. Khasanova, L.U. Dzhemileva, E.K. Khusnutdinova, 2017, published in Genetika, 2017, Vol. 53, No. 5, pp. 517–525.

REVIEW
AND THEORETICAL ARTICLES

Genetic Aspects of Keratoconus Development

M. M. Bikbov[a], E. L. Usubov[a], K. Kh. Oganisyan[a, *], S. L. Lobov[b], R. R. Khasanova[d], L. U. Dzhemileva[b, c, **], and E. K. Khusnutdinova[b, d]

[a] *Ufa Eye Research Institute, Academy of Sciences of the Republic of Bashkortostan, Ufa, 450008 Russia*
[b] *Institute of Biochemistry and Genetics, Ufa Scientific Center, Russian Academy of Sciences, Ufa, 450054 Russia*
[c] *Department of Human Reproductive Health, Bashkir State Medical University, Ufa, 450008 Russia*
[d] *Department of Genetics and Fundamental Medicine, Bashkir State University, Ufa, 450076 Russia*
**e-mail: oganisyan.karine@gmail.com*
***e-mail: dzhemilev@mail.ru*

Received May 5, 2016; in final form, June 6, 2016

Abstract—Keratoconus (KC) is the most common form of keratoectasia characterized by changes in corneal topography and its thinning, stretching, and protrusion. The hereditary or genetic theory of keratoconus development is widely recognized. To date, a large number of candidate genes have been investigated in patients with KC. One of the most important of them are the gene encoding a homeodomain-containing protein that belongs to the subfamily of paired-like homeodomain proteins (*VSX1*), superoxidedismutase 1 (*SOD1*) gene, and the gene of lysyloxidase (*LOX*). The linkage analysis reveals over 17 chromosomal regions mutations in which can lead to the development of KC. In families with a hereditary form of keratoconus by GWAS analysis, the association of central corneal thickness (CCT) with a number of genetic loci is revealed. Thus, diverse results of genetic studies and a large number of identified chromosomal regions associated with keratoconus, firstly, show marked genetic heterogeneity of the disease and, secondly, are associated with challenges in DNA diagnosis of this disease. However, there are prerequisites that keratoconus belongs to both hereditary and genetically caused diseases and identified genetic variants are specific both to individual populations and to certain ethnic groups in general.

Keywords: keratoconus, genetics, etiology, cornea, ectasia
DOI: 10.1134/S1022795417040020

INTRODUCTION

Keratoconus (KC) is the most common form of keratectasia characterized by changes in corneal topography and its thinning, stretching, and protrusion. The stroma of the cornea is represented by different types of collagen fibers, keratocytes, and glycoprotein matrix. Quantitative and qualitative changes in collagen fibrils lead to their reorganization and change in the biomechanical properties of the cornea. Keratoconus is bilateral, manifesting itself in the second and third decades of life, and leads to a significant reduction in vision. Today the hereditary or genetic theory of the disease is generally recognized. The familial cases of keratoconus, its frequent combination with a number of hereditary diseases and syndromes, and the peculiarities of keratoconus in monozygotic and dizygotic twins serve as irrefutable proof of it [1–6].

The frequency of keratoconus varies considerably—from 0.3 per 100000 population in Russia up to 2300 per 100000 in Central India (0.0003–2.3%). Hereditary forms occur in 5–23.5% of cases [7–11]. For example, studies in the northeastern region of Iran (Mashhad) and in Lebanon (Beirut) showed that the genetic etiology of keratoconus was confirmed in 11.4 and 12.1% of families, respectively [12, 13]. According to a multicenter prospective study CLERK (United States, 1998), familial keratoconus occurs in 13.5% of cases [14]. Published in 2005, data from population studies conducted in Saudi Arabia showed that hereditary forms of keratectasia occurred in 16% of cases [15]. Among the population of Israel and New Zealand, this figure was much higher and amounted to 21.74 and 23.5%, respectively [16, 17].

GENETIC DIVERSITY OF KERATOCONUS HEREDITARY FORMS

To date, a large number of candidate genes in patients with KC have been investigated. One of the most important of them are the gene encoding a homeodomain-containing protein that belongs to the subfamily of paired-like homeodomain proteins associated with the development of eyes and craniofacial region (*VSX1*) and the superoxide dismutase 1 gene (*SOD1*).

The total length of the *VSX1* gene, containing five exons, is about 10.65 kb [18]. It was mapped in 2000 within the short arm of chromosome 20 (20p11.2) [19, 20]. The *VSX1* gene encodes a homeodomain-containing protein, visual system homeobox 1 (VSX1), consisting of 365 amino acids, and it includes a paired-like domain. The VSX1 protein is involved in the processes of formation and development of the eyeball structures and craniofacial region. Its intense expression was detected in embryonic cells, retinal cells, and keratinocytes. The structure of the human VSX1 protein is 55% identical to the same protein of zebrafish fish and 35% identical to mouse protein [19–23].

Heon et al. [21] reported the presence of a heterogeneous missense mutation in the *VSX1* gene in a patient with a sporadic form of keratoconus, leading to an arginine to tryptophan substitution at position 166 (p.R166W). Using the method of shift of electrophoretic activity of protein fragments, the reduction in the DNA-binding activity of the mutant protein was shown. In addition, missense mutation in the *VSX1* gene leading to glycine to asparagine substitution at position 160 (p.G160D) in the CVC domain of the protein was found in four siblings with the developed form of posterior polymorphous corneal dystrophy (PPCD). In one member of the family with advanced PPCD together with the p.G160D mutation, the missense mutation leading to the substitution of proline to arginine at position 247 (p.P247R) in the CVC domain of VSX1 was also present. These mutations were absent from 200 healthy individuals without keratoconus in the control group [21]. Later, in 2005, the results of Bisceglia et al. were published [24], which confirmed the presence of a heterogeneous p.G160D missense mutation in an Italian family with keratoconus without concomitant PPCD. The transition (c.323T > C) in the first exon of the *VSX1* gene leading to the substitution of leucine to proline in the seventeenth position of VSX1 protein (p.L17P) was detected in three families with keratoconus. This mutation, along with the previously detected ones (p.D144E, p.G160D, and p.P247R), occurred in 8.7% of cases and was completely absent from the control group. The authors hypothesized that mutations in the *VSX1* gene play an important role in the development of autosomal dominant forms of keratoconus [24]. Dash et al. found a missense mutation leading to an amino acid substitution (p.G160D) in patients with a sporadic form of keratoconus, which was also absent from 100 individuals from the control group. In a study of 222 patients with keratoconus from Italy, the nucleotide substitution c.715G > C leading to the substitution of arginine to glycine at position 239 (p.G239R) in the CVC domain of VSX1 protein was found [25]. Possibly, this mutation leads to a change in the secondary or tertiary protein structure. In 4.1% of cases, missense mutations leading to amino acid substitutions were found: p.L17P, p.D144E, and p.P247R. However, according to Y.G. Tang et al. [26], VSX1 protein expression abnormality is the cause of the disease only in some populations; to determine the role of mutations in the development of keratoconus, further research is required.

Many studies have shown the role of oxidative stress in the development of keratoconus [27–35]. The cornea is exposed to various light spectra, including ultraviolet. Ultraviolet light is a triggering factor in the development of oxidative stress, which induces the release of free radicals. However, a number of enzymes with antioxidative activity are present in a healthy cornea, in particular, superoxide dismutase, glutathione reductase, and glutathione peroxidase, preventing the destruction of the membrane and mitochondria of keratocytes by free radicals. The *SOD1* gene, consisting of five exons, encodes a large group of antioxidant enzymes. These antioxidants in normal conditions support the permanent concentration of superoxide radicals at a certain level, thereby protecting the cell structures from the damaging effects of oxygen radicals themselves and from occurrence of hydroxyl radicals, which may be formed from oxygen and hydroxide [36]. Over 50 mutations are described in all exons of the *SOD1* gene (they are mainly represented by point mutations and less commonly by insertions and deletions) [37–39] and associated with the development of amyotrophic lateral sclerosis. Some of the mutations are described in only some families; others are major in certain populations. However, there is no information about the combination of this pathology with KC. Frequent combination of Down syndrome and KC has been described by many authors [40, 41]. Approximately 15% of Caucasoid patients with Down syndrome suffer from KC [42]. The intensification of processes of oxidative damage and the increase in the concentration of superoxide radicals occur in Down syndrome.

A series of papers was devoted to the search for mutations in the *SOD1* gene in patients with KC [43–47]. Udar et al. [46] in 2006 conducted an analysis of the exon–intron structure of the *SOD1* gene in 15 unrelated patients with familial keratoconus. The splice site mutation in the second intron was found in three patients with KC. This mutation was absent in 156 individuals of the control group. Later, in 2011, Bonis et al. [44] published data of a genetic study of a population of patients from Italy and confirmed the presence of IVS2 +50del7 deletion in the second intron of the *SOD1* gene in two patients with the sporadic form of the disease. The mutation was absent in 200 individuals of the control group. However, in the study of a population of patients with KC from Slovenia, the aforementioned mutation was absent in both patients and control individuals [43]. Similar results were obtained by Samira Sae-Rad et al. [48] in a population of Iranian patients, where three previously undescribed SNP variants (g.4886G > A, g.4990C > G, and g.9061T > A) in the *SOD1* gene were

described. The Iranian researchers demonstrated that the expression of the *SOD1* gene in the cornea of patients with KC was slightly lower than in the cornea of healthy individuals, but the difference was not statistically significant [49]. But when looking for the IVS2 +50del7 deletion in individuals from Greece in patients with KC, the heterozygous variant of this mutation was observed more frequently than in healthy individuals ($P = 0.002$). The homozygous mutation was not detected in any of the studied groups [50]. Also among patients from Saudi Arabia, the IVS2 +50del7 mutation in the *SOD1* gene was not detected in any of the examined individuals [51].

Another candidate gene involved in the pathogenesis of KC is the *LOX* gene. Lysyl oxidase (LOX) is the extracellular Cu-dependent enzyme that catalyzes the removal of NH_2 groups of lysine and oxylysine residues in nonspiral NH_2- and COOH-terminal regions and simultaneously oxidizes ε-carbon in CHO (aldehyde). This produces δ-semialdehyde of α-aminoadipic acid (allizin) and δ-tolualdehyde of δ-oxy-α-aminoadipic acid, respectively. The cross-links make only the δ-semialdehyde of α-aminoadipic acid and δ-semialdehyde of δ-oxy-α-aminoadipic acid [52]. The enzyme specifically deaminates the lysine residues of polypeptide chain, showing no activity in relation to free lysine and being inhibited by β-aminopropionitrile. In fibroblast cell culture in vitro, the cross-links in collagen monomers do not occur if β-aminopropionitrile is added to the medium or the medium is copper poor. Hamalainen et al. [53] mapped the gene encoding LOX on chromosome 5 at 5q23.3-q31.2. LOX protein deficiency is detected in the following diseases: Ehlers-Danlos syndrome type IX (OMIM 304150) and type V (OMIM 305200) and Menkes disease (OMIM 309400). Recently, the role of the *LOX* gene in suppression of carcinogenesis, in the aging process of cells, and in chemotaxis [54, 55] was demonstrated, and also its association with atherosclerosis, dissecting aortic aneurysm, with the development of hypertrophic scars, and with proliferatinoy retinopathy was revealed [56, 57].

Currently, the role of the *LOX* gene in the development of keratoconus is actively being investigated. For the first time, the immunohistochemical studies of lysyl oxidase were performed by Nielson et al. [58] in 2003, according to which in patients with keratoconus the gene expression was increased in the diseased cornea. However, Dudakova et al. obtained controversial results, confirmed in subsequent studies: in a group of patients with keratoconus, *LOX* gene expression was decreased [59] and the expression level was inversely related to the stage of keratoconus. Rohit Shetty et al., besides *LOX*, also examined the expression of the *COL IA1* (type I collagen), *COL IVA1* (type IV collagen), *MMP9* (metalloproteinase 9), and *IL6* (interleukin 6) genes in the epithelial substrate obtained from cornea of patients with keratoconus [60]. The immunocytochemical analysis revealed the increased MMP9 and IL6 activity together with the reduced level of lysyl oxidase in epithelial cells and type IV collagen in the basement membrane of epithelium.

As a result of the search for *LOX* gene mutations in an Italian population, two polymorphic variants were identified—rs41407546 (c.476C > A, p.P159Q) and rs1800449 (c.473G > A, R158Q). The rs41407546*A*C (c.476C > A, p.P159Q) genotype was detected in five patients with the sporadic form of keratoconus. The rs41407546*G*A (c.473G > A, R158Q) genotype was identified in 78 patients; the frequency of G allele (0.844) and A allele (0.155) was comparable with the distribution of allele frequencies of the HapMap project [61]. Studies in patients with the sporadic form of keratoconus from a Czech population revealed the presence of a polymorphic variant rs2956540 (c.1035 + 528C > G, n.312-1830G > C, and g.122073485G > C), whose allele turned out to be associated with the sporadic form of KC [62].

By linkage analysis, more than 17 chromosomal regions were determined in which mutations can lead to the development of KC (table), confirming the genetic heterogeneity of this disease [63].

According to three independent studies, only some genomic regions were further obtained, in particular, chromosomal region 5q21.2 [26, 62]. Recently, the role of this region was confirmed by linkage analysis (high density SNPs). Similar results of the three studies may indicate the possible presence of a common locus in chromosome 5, which plays a role in the KC pathogenesis [74].

The screening for mutations in KC candidate genes, located at 16q22.3-q23.1 (OMIM 608932), 3p14-q13 (OMIM 608586), 13q32, 2p24 (OMIM 609271), 5q14.3-q21.1, 15q23-24, 20q12, 20p11.2 (*VSX1*), and 21q22 (*SOD1*), was carried out in 18 families of patients from Ecuador with autosomal dominant KC form. The previously identified 13q32 region was interpreted as linked with this disease [68]. Czugala et al. [75] conducted a search for possible mutations in candidate genes in the 13q32 region: *MBNL1*, *IPO5*, *FARP1*, *RNF113B*, *STK24*, *DOCK9*, *ZIC5*, and *ZIC2*. The researchers identified a mutation c.2262A > C (p. Gln754His) in the *DOCK9* gene (dedicator of cytokinesis 9) only in patients with KC. The mutation was absent in the control group of healthy individuals. This gene encodes a member of a family of proteins involved in cytokinesis (DOCK) of atypical factors of guanine nucleotides metabolism. The factors of metabolism of guanine nucleotides interact with small GTPases and the components of intracellular signaling networks. This protein from the group of C DOCK proteins plays a role in reorganization of the actin cytoskeleton by activating Rho GTPases Cdc42 and Rac1, actively expressed in the cornea [68, 76]. To determine the functional significance of the mutation identified by Czugala et al. [75], the influence of it on

Genetic loci associated with keratoconus

Region	Inheritance	Region of residence of patients	Reference
1p36.23-36.21	Autosomal dominant	Australia	K.P. Burdon et al., 2008 [64]
2p24	–	European, Arab, and African families	H. Hutchings et al., 2005 [65]
3p14-q13	Autosomal dominant	Italy	F. Brancati et al., 2004 [66]
5q14.3-q21.1	Autosomal dominant	Italy (south)	L. Bisceglia et al., 2009 [61] Y.G. Tang et al., 2005 [26]
5q23.2	–	Spain	X. Li et al., 2006 [67]
5q32-q33	–	Italy	L. Bisceglia et al., 2009 [61]
8q13.1-q21.11	Autosomal dominant	Australia	K.P. Burdon et al., 2008 [64]
9q34	–	Spain	X. Li et al., 2006 [67]
13q32	Autosomal dominant	Ecuador	M. Gajecka et al., 2009 [68]
14q11.2	–	Italy (south)	L. Bisceglia et al., 2009 [61]
14q11.2	–	Spain	X. Li et al., 2006 [67]
14q24.3	–	Europe, Asia, and Africa	P. Liskova et al., 2010 [69]
15q2.32	–	Italy (south)	L. Bisceglia et al., 2009 [61]
15q22.33-24.2	Autosomal dominant	Ireland	A.E. Hughes et al., 2003 [70]
16q22.3-q23.1	Autosomal dominant	Finland	H. Tyynismaa et al., 2002 [71]
17q13	Autosomal recessive	Pakistan	A. Hameed et al., 2000 [72]
20q12	Autosomal dominant	Australia	J. Fullerton et al., 2002 [73]

the splicing process in vitro was investigated. It was found that the c.2262A > C variant in the twentieth exon of dedicator of cytokinesis 9 gene led to the disruption of splicing and formation of two protein isoforms. Changing the balance between these isoforms, with a predominance of a defective form, may lead to functional impairment of the domain of DOCK proteins as activators of Cdc42 and development of KC.

ROLE OF MiRNAs IN THE ETIOLOGY OF KERATOCONUS

Mutation r.57c > u was identified in the miR-184 gene. MiRNAs are short (19–25 nucleotides) noncoding, regulatory RNAs that are involved in the suppression of gene activity, complementarily binding to 3'-UTR of mRNA of target genes according to the principle of complete or partial complementarity; they regulate gene expression post-transcriptionally and induce degradation of mRNA inhibition of translation [77]. This mechanism allows miRNAs to be involved in the regulation of many cellular processes, including growth, differentiation, apoptosis, proliferation, stress response, metabolism, and insulin secretion [78]. The family of miRNA genes occupies a little over 1% of the human genome, but regulates the expression of almost a third of all genes at the post-transcriptional level, at the same time being the most conservative in sequences and mechanisms of expression. The key role of miRNAs in the imbalance of proliferation, differentiation, and programmed cell death during the development of various diseases, including cancer, has been revealed. Significant tissue specificity of expression of miRNAs leads to probably the variability of the protein profile in various organs and systems. The miR-184 gene (encoding *MIR184* [MIM 613146]) is expressed in the basal membrane of the epithelium of the cornea and the lens and is involved in the regulation of protein level in these tissues. One of the functions of *MIR184* is competitive inhibition of *MIR-205* binding to mRNA of inositol polyphosphate phosphatase-like 1 gene *INPPL1* (MIM 600829). Thus, INPPL1 expression increases indirectly, which maintains the level of the phosphorylated AKT (RAC-alpha serine/threonine-protein kinase, protein kinase B alpha) and BAD (BCL-2-associated death promoter). The latter, in turn, participate in the regulation of apoptosis of corneal cells [79]. Hughes et al. [80] found the r.57c > T transition in the *MIR-184* gene in patients with familial keratoconus and early-onset anterior polar cataract from Northern Ireland. The authors conducted a search for possible mutations in more than 20 candi-

date genes in the 15q22-q25 region 5 Mb in size [70], and the mutation was found only in the *MIR184* gene. Subsequently, Lechner et al. [81] identified two substitution-type mutations in *MIR184* (+3A > G and +8C > A) in two patients with sporadic forms of keratoconus. Similar results were obtained by Y. Bykhovskaya et al. [74] during the examination of familial cases of keratoconus. However, no mutations in this gene were found among 134 patients with keratoconus but without concomitant ocular pathology in Saudi Arabia [82].

GENOME-WIDE ASSOCIATION IN KERATOCONUS RESEARCH

The method of genome-wide association study (GWAS) to date is widely used in ophthalmic genetics and provides information about the genetic relationship of symptoms and diseases. In families with a hereditary form of keratoconus, GWAS revealed association of central corneal thickness (CCT) with a number of genetic loci—*ZNF469*, *COL5A1*, *RXRA-COL5A1*, *COL8A2*, *AKAP13*, *AVGR8*, *FOXO1*, *FNDC3B*, *TJP1*, *NR3C2*, *LRRK1*, *FDF9-SGCG*, *LCN12-PTGDS*, *ADAMTS6*, *CHSY1*, *HS3ST3B1-PMP22*, *GLT8D2*, *SMAD3*, *VKORC1L1*, *COL4A3*, *FAM46A-IBTK*, *LPAR1*, *ARID5B*, *TBL1XR1-KCNMB2*, *ARHGAP20-POU2AF1*, *C7ORF42*, *MPDZ-NF1B*, *USP37*, *GPR15*, and *TIPARP* [83–86]. Several genetic loci and regions associated with the CCT of the region, including *FOXO1* and *FNDC3B*, are risk factors for the development of keratoconus, which indicates the role of collagen and extracellular matrix in the pathogenesis of the disease [85]. Currently, the results from two GWAS are available, according to which missense mutations in the *ZNF469* gene were identified in 12.5 and 23.3% of patients with sporadic forms of keratoconus in the United Kingdom and New Zealand, which may indirectly suggest the possible involvement of the *ZNF469* gene in the development of keratoconus [87, 88].

In 2014, Y. Liu et al. showed multiple deletions in patients with hereditary forms of keratoconus in chromosomal regions associated with the CCT, including *RXRA-COL5A1* and *HS3ST3B1-PMP22*, as well as with refraction errors in the *GRIA4* region [89].

In 2012, Li et al. [90] conducted a GWAS study for 222 patients with KC. They reported on the association of the genomic region adjacent to the *RAB3GAP1* gene (2q21.3), although the subsequent replication analysis appeared unreliable. This association was also found by Bae et al. [91]. The *RAB3GAP1* gene regulates the activity of RAB3 protein, activating subunit 1 of RAB3GTPase catalytic enzyme. The encoded protein forms a heterodimer with the noncatalytic subunit, specifically regulating the activity of the representatives of the Rab3 subfamily of small proteins. This protein mediates hydrolysis of GTP, bound with Rab3, to GDP. Interestingly, the mutation in this gene is associated with Warburg syndrome—a rare autosomal recessive disorder, which manifests itself with microphthalmia, microcornea, congenital cataract, and optic atrophy [92–95].

Burdon et al. [96] conducted a GWAS study among Australian patients with keratoconus. The association with the gene of hepatocyte growth factor (*HGF*) had the greatest importance. This association was also found by Sahebjada et al. [97] in patients with KC from an Australian population ($P < 0.003$). The *HGF* gene stimulates proliferation of some types of epithelial cells, vascular endothelial cells, and melanocytes. According to other authors, the *HGF* gene was associated with refraction errors in Caucasoid and Chinese populations [98–100].

No copy number variations (CNV) have been found in patients with sporadic forms of keratoconus, indicating that this mechanism does not participate in keratoconus pathogenesis [100].

CONCLUSIONS

Thus, heterogeneous results of genetic studies and a large number of identified chromosomal regions linked with keratoconus, firstly, show marked genetic heterogeneity of the disease and, secondly, are associated with challenges in DNA diagnosis of this disease. Nevertheless, there are marked prerequisites that keratoconus relates to both hereditary and genetically caused diseases and the identified genetic variants are specific both to individual populations and to certain ethnic groups in general. Identification of genetic variants the most important and universal for different groups of patients will help to develop methods for early diagnosis and effective prevention of this disease.

REFERENCES

1. Sugar, J. and Macsai, M.S., What causes keratoconus? *Cornea*, 2012, vol. 31, no. 6, pp. 716—719. doi 10.1097/ICO.0b013e31823f8c72
2. Edwards, M., The genetics of keratoconus, *Clin. Exp. Ophthalmol.*, 2001, vol. 29, no. 6, pp. 345—351.
3. Bikbov, M.M. and Bikbova, G.M., *Ektazii rogovitsy (patogenez, patomorfologiya, klinika, diagnostika, lechenie)* (Corneal Ectasia (Pathogenesis, Pathomorphology, Clinical Picture, Diagnosis, Treatment)), Moscow: Oftal'mologiya, 2011.
4. Oganisyan, K.Kh., Genetic aspects of keratoconus (review), *Sbornik nauchnykh trudov Mezhdunarodnoi nauchno-prakticheskoi konferentzii po oftal'mokhirurgii "Vostok-Zapad"* (Proceedings of International Scientific and Practical Conference for Ophthalmic Surgery "East—West"), Ufa, 2013, p. 98.
5. Bikbov, M.M., Surkova, V.K., and Oganisyan, K.Kh., Keratoconus as a manifestation of connective tissue dysplasia, *Oftal'mologiya*, 2015, vol. 12, no. 1, pp. 4—7.
6. Mglinets, V.A., Molecular genetics of development of cornea, *Russ. J. Genet.*, 2015, vol. 51, no. 1, pp. 1—8.

7. Surkova, V.K. and Oganisyan, K.Kh., Epidemiology of primary corneal ectasia, *Vestn. Orenburg. Gos. Univ.*, 2015, vol. 187, no. 12, pp. 234—238.
8. Davidson, A.E., Hayes, S., Hardcastle, A.J., et al., The pathogenesis of keratoconus, *Eye*, 2014, vol. 28, no. 2, pp. 189—195. doi 10.1038/eye.2013.278
9. Abu-Amero, K.K., Al-Muammar, A.M., and Kondkar, A.A., Genetics of keratoconus: where do we stand?, *J. Ophthalmol.*, 2014, vol. 2014. doi 10.1155/2014/641708.641708
10. Wang, Y., Rabinowitz, Y.S., Rotter, J.I., et al., Genetic epidemiological study of keratoconus: evidence for major gene determination, *Am. J. Med. Genet.*, 2000, vol. 93, no. 5, pp. 403—409.
11. Avetisov, S.E., Novikov, I.A., and Pateyuk, A.S., Keratoconus: ethiological factors and associated symptoms, *Vestn. Oftal'mol.*, 2014, no. 4, pp. 110—113.
12. Waked, N., Fayad, A.M., Fadlallah, A., et al., Keratoconus screening in a Lebanese students' population, *J. Fr. Ophtalmol.*, 2012, vol. 35, no. 1, pp. 23—29.
13. Hashemi, H., Beiranvand, A., and Khabazkhoob, M., Prevalence of keratoconus in a population-based study in Shahroud, *Cornea*, 2013, vol. 32, no. 11, pp. 1441—1445.
14. Zadnik, K., Barr, J.T., and Edrington, T.B., Baseline findings in the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) study, *Invest. Ophthalmol. Vis. Sci.,* 1998, vol. 39, no. 13, pp. 2537—2546.
15. Assiri, A.A., Yousuf, B.I., Quantock, A.J., et al., Incidence and severity of keratoconus in Asir province, Saudi Arabia, *Br. J. Ophthalmol.*, 2005, vol. 89, no. 11, pp. 1403—1406.
16. Millodot, M., Shneor, E., Albou, S., et al., Prevalence and associated factors of keratoconus in Jerusalem: a cross-sectional study, *Ophthalmic Epidemiol.*, 2011, vol. 18, no. 2, pp. 91—97.
17. Owens, H. and Gamble, G., A profile of keratoconus in New Zealand, *Cornea*, 2003, vol. 22, no. 2, pp. 122—125.
18. Hosseini, S.M., Herd, S., Vincent, A.L., et al., Genetic analysis of chromosome 20-related posterior polymorphous corneal dystrophy: genetic heterogeneity and exclusion of three candidate genes, *Mol. Vis.*, 2008, vol. 14, pp. 71—80.
19. Semina, E.V. Mintz-Hittner, H.A., et al., Isolation and characterization of a novel human paired-like homeodomain-containing transcription factor gene, VSX1, expressed in ocular tissues, *Genomics*, 2000, vol. 63, no. 2, pp. 289—293. doi 10.1006/geno.1999.6093
20. Hayashi, T., Huang, J., and Deeb, S.S., RINX(VSX1), a novel homeobox gene expressed in the inner nuclear layer of the adult retina, *Genomics*, 2000, vol. 67, no. 2, pp. 128—139. doi 10.1006/geno.2000.6248
21. Héon, E., Greenberg, A., Kopp, K.Kl., et al., VSX1: a gene for posterior polymorphous dystrophy and keratoconus, *Hum. Mol. Genet.*, 2002, vol. 11, no. 9, pp. 1029—1036. doi 10.1093/hmg/11.9.1029
22. Ohtoshi, A., Wang, S.W., and Maeda, H., Regulation of retinal cone bipolar cell differentiation and photopic vision by the CVC homeobox gene Vsx1, *Curr. Biol.*, 2004, vol. 14, no. 6, pp. 530—536. doi 10.1016/j.cub.2004.02.027
23. Watson, T. and Chow, R.L., Absence of Vsx1 expression in the normal and damaged mouse cornea, *Mol. Vis.*, 2011, vol. 17, pp. 737—744.
24. 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, vol. 46, pp. 39—45. doi 10.1167/iovs.04-0533
25. Dash, D.P., George, S., O'Prey, D., et al., Mutational screening of VSX1 in keratoconus patients from the European population, *Eye*, 2010, vol. 24, pp. 1085—1092.
26. Tang, Y.G., Rabinowitz, Y.S., and Taylor, K.D., Genomewide linkage scan in a multigeneration Caucasian pedigree identifies a novel locus for keratoconus on chromosome 5q14.3-q21.1, *Genet. Med.*, 2005, vol. 7, no. 6, pp. 397—405. doi 10.1097/01.gim.0000170772.41860.54
27. Romero-Jiménez, M., Santodomingo-Rubido, J., and Wolffsohn, J.S., Keratoconus: a review, *Contact Lens Anterior Eye*, 2010, vol. 33, no. 4, pp. 157—166. doi 10.1016/j.clae.2010.04.006
28. Stabuc-Silih, M., Strazisar, M., Ravnik-Glavac, M., et al., Genetics and clinical characteristics of keratoconus, *Acta Dermatovenerol. Alp., Pannonica, Adriat.*, 2010, vol. 19, no. 2, pp. 3—10.
29. Gondhowiardjo, T.D. and van Haeringen, N.J., Corneal aldehyde dehydrogenase, glutathione reductase, and glutathione S-transferase in pathologic corneas, *Cornea*, 1993, vol. 12, pp. 310—314.
30. Kenney, M.C., Chwa, M., and Atilano, S.R., Increased levels of catalase and cathepsin V/L2 but decreased TIMP-1 in keratoconus corneas: evidence that oxidative stress plays a role in this disorder, *Invest. Ophthalmol. Vis. Sci.,* 2005, vol. 46, pp. 823—832.
31. Behndig, A., Karlsson, K., Johansson, B.O., et al., Superoxide dismutase isoenzymes in the normal and diseased human cornea, *Invest. Ophthalmol. Vis. Sci.*, 2001, vol. 42, pp. 2293—2296.
32. Buddi, R., Lin, B., Atilano, S.R., Zorapapel, N.C., et al., Evidence of oxidative stress in human corneal diseases, *J. Histochem. Cytochem.*, 2002, vol. 50, pp. 341—351.
33. Chwa, M., Atilano, S.R., Reddy, V., et al., Increased stress-induced generation of reactive oxygen species and apoptosis in human keratoconus fibroblasts, *Invest. Ophthalmol. Vis. Sci.*, 2006, vol. 47, pp. 1902—1910.
34. Chwa, M., Atilano, S.R., and Hertzog, D., Hypersensitive response to oxidative stress in keratoconus corneal fibroblasts, *Invest. Ophthalmol. Vis. Sci.*, 2008, vol. 49, pp. 4361—4369.
35. Shoham, A., Hadziahmetovic, M., Dunaief, J.L., et al., Oxidative stress in diseases of the human cornea, *Free Radic. Biol. Med.*, 2008, vol. 45, pp. 1047—1055.

36. Noor, R., Mittal, S., and Iqbal, J., Superoxide dismutase—applications and relevance to human diseases, *Med. Sci. Monitor.*, 2002, vol. 8, no. 9, pp. 210—215.

37. Lewis, P. and Rowland Neil, A., Amyotrophic lateral sclerosis, *N. Engl. J. Med.*, 2001, vol. 344, no. 22, pp. 1688—1700.

38. Majoor-Krakauer, D. and Willems, P.J., Genetic epidemiology of amyotrophic lateral sclerosis, *Clin. Genet.*, 2003, vol. 63, no. 2, pp. 83—101.

39. Ticozzi, N., Tiloca, C., Morelli, C., et al., Genetics of familial amyotrophic lateral sclerosis, *Arch. Ital. Biol.*, 2011, vol. 149, pp. 65—82.

40. Stoiber, J., Muss, W., and Ruckhofer, J., Acute keratoconus with perforation in a patient with Down's syndrome, *Br. J. Ophthalmol.*, 2003, vol. 87, p. 120.

41. Soriano, L., Munoz Calvo, M., and Pozo Roman, J., Graves disease in patients with Down's syndrome, *An. Pediatr.*, 2003, vol. 58, pp. 63—66.

42. Rabinowitz, Y.S., Keratoconus, *Surv. Ophthalmol.*, 1998, vol. 42, pp. 297—319.

43. Štabuc-Šilih, M., Stražišar, M., Hawlina, M., et al., Absence of pathogenic mutations in VSX1 and SOD1 genes in patients with keratoconus, *Cornea*, 2010, vol. 29, no. 2, pp. 172—176. doi 10.1097/ICO.0b013e3181aebf7a

44. Bonis, P., Laborante, A., and Pizzicoli, C., Mutational screening of VSX1, SPARC, SOD1, LOX, and TIMP3 in keratoconus, *Mol. Vision*, 2011, vol. 17, pp. 2482—2494.

45. Sussan, T.E., Yang, A., Li, F., et al., Trisomy represses ApcMin-mediated tumours in mouse models of Down's syndrome, *Nature*, 2008, vol. 451, pp. 73—75. doi 10.1038/nature06446

46. Udar, N., Atilano, S.R., and Brown, D.J., SOD1: a candidate gene for keratoconus, *Invest. Ophthalmol. Vis. Sci.*, 2006, vol. 47, no. 8, pp. 3345—3351. doi 10.1167/iovs.05-1500

47. Udar, N., Atilano, S.R., Small, K., et al., SOD1 haplotypes in familial keratoconus, *Cornea*, 2009, vol. 28, no. 8, pp. 902—907. doi 10.1097/ICO.0b013e3181983a0c

48. Saee-Rad, S.1., Hashemi, H., Miraftab, M., et al., Mutation analysis of VSX1 and SOD1 in Iranian patients with keratoconus, *Mol. Vis.*, 2011, vol. 17, pp. 3128—3136.

49. Saee-Rad, S.1., Raoofian, R., Mahbod, M., et al., Analysis of superoxide dismutase 1, dual-specificity phosphatase 1, and transforming growth factor, beta 1 genes expression in keratoconic and non-keratoconic corneas, *Mol. Vis.*, 2013, vol. 19, pp. 2501—2507.

50. Moschos, M., Kokolakis, N., Gazouli, M., et al., Polymorphism analysis of *VSX1* and *SOD1* genes in Greek patients with keratoconus, *Ophthalmic Genet.*, 2015, vol. 36, no. 3, pp. 213—217. doi 10.3109/13816810.2013.843712

51. Al-Muammar, A.M., Kalantan, H., and Azad, T.A., Analysis of the SOD1 gene in keratoconus patients from Saudi Arabia, *Ophthalmic Genet.*, 2015, vol. 36, no. 4, pp. 373—375. doi 10.3109/13816810.2014.889173

52. Kok, Y.O., Ling, G.F., and Loon, S.C., Review: keratoconus in Asia, *Cornea*, 2012, vol. 31, no. 5, pp. 581—593. doi 10.1097/ico.0b013e31820cd61d

53. Hamalainen, E.R., Jones, T.A., Sheer, D., et al., Molecular cloning of human lysyl oxidase and assignment of the gene to chromosome 5q23.3-31.2, *Genomics*, 1991, vol. 11, pp. 508—516.

54. Mäki, J.M., Lysyl oxidases in mammalian development and certain pathological conditions, *Histol. Histopathol.*, 2009, vol. 24, pp. 651—660.

55. Cano, A., Santamaria, P.G., and Moreno-Bueno, G., LOXL2 in epithelial cell plasticity and tumor progression, *Future Oncol.*, 2012, vol. 8, pp. 1095—1080.

56. Coral, K., Angayarkanni, N., Madhavan, J., et al., Lysyl oxidase activity in the ocular tissues and the role of LOX in proliferative diabetic retinopathy and rhegmatogenous retinal detachment, *Invest. Ophthalmol. Vis. Sci.*, 2008, vol. 49, pp. 4746—4752.

57. Lucero, H.A1. and Kagan, H.M., Lysyl oxidase: an oxidative enzyme and effector of cell function, *Cell Mol. Life Sci.*, 2006, vol. 63, pp. 2304—2316.

58. Nielsen, K., Birkenkamp-Demtroder, K., Ehlers, N., et al., Identification of differentially expressed genes in keratoconus epithelium analyzed on microarrays, *Invest. Ophthalmol. Vis. Sci.*, 2003, vol. 44, pp. 2466—2476.

59. Dudakova, L., Liskova, P., Trojek, T., et al., Changes in lysyl oxidase (LOX) distribution and its decreased activity in keratoconus corneas, *Exp. Eye Res.*, 2012, vol. 104, pp. 74—81.

60. Shetty, R., Sathyanarayanamoorthy, A., Ramachandra, R.A., et al., Attenuation of lysyl oxidase and collagen gene expression in keratoconus patient corneal epithelium corresponds to disease severity, *Mol. Vis.*, 2015, vol. 21, pp. 12—25.

61. Bisceglia, L., de Bonis, P., and Pizzicoli, C., Linkage analysis in keratoconus: replication of locus 5q21.2 and identification of other suggestive loci, *Invest. Ophthalmol. Vis. Sci.*, 2009, vol. 50, no. 3, pp. 1081—1086. doi 10.1167/iovs.08-2382

62. Dudakova, L., Palos, M., Jirsova, K., et al., Validation of rs2956540:G>C and rs3735520:G>A association with keratoconus in a population of European descent, *Eur. J. Hum. Genet.*, 2015, vol. 23, no. 11, pp. 1581—1583. doi 10.1038/ejhg.2015.28

63. Wheeler, J., Hauser, M.A., Afshari, N.A., et al., The genetics of keratoconus: a review, *Reprod. Syst. Sex. Disord.*, 2012, suppl. 6, p. 001. doi 10.4172/2161-038X.S6-001

64. Burdon, K.P., Coster, D.J., Charlesworth, J.C., et al., Apparent autosomal dominant keratoconus in a large Australian pedigree accounted for by digenic inheritance of two novel loci, *Hum. Genet.*, 2008, vol. 124, no. 4, pp. 379—386. doi 10.1007/s00439-008-0555-z

65. Hutchings, H., Ginisty, H., and Gallo, M., Identification of a new locus for isolated familial keratoconus at 2p24, *J. Med. Genet.*, 2005, vol. 42, no. 1, pp. 88—94.

66. Brancati, F., Valente, E.M., and Sarkozy, A., A locus for autosomal dominant keratoconus maps to human chromosome 3p14-q13, *J. Med. Genet.*, 2004, vol. 41, pp. 188—192.

67. Li, X., Rabinowitz, Y.S., and Tang, Y.G., Two-stage genome-wide linkage scan in keratoconus sib pair families, *Invest. Ophthalmol. Vis. Sci.*, 2006, vol. 47, no. 9, pp. 3791—3795. doi 10.1167/iovs.06-0214

68. 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, vol. 50, no. 4, pp. 1531—1539. doi 10.1167/iovs.08-2173

69. Liskova, P., Hysi, P.G., Waseem, N., et al., Evidence for keratoconus susceptibility locus on chromosome 14: a genome-wide linkage screen using single-nucleotide polymorphism markers, *Arch. Ophthalmol.*, 2010, vol. 128, pp. 1191—1195.

70. Hughes, A.E., Dash, D.P., Jackson, A.J., et al., Familial keratoconus with cataract: linkage to the long arm of chromosome 15 and exclusion of candidate genes, *Invest. Ophthalmol. Vis. Sci.*, 2003, vol. 44, no. 12, pp. 5063—5066. doi 10.1167/iovs.03-0399

71. Tyynismaa, H., Sistonen, P., Tuupanen, S., et al., A locus for autosomal dominant keratoconus: linkage to 16q22.3-q23.1 in Finnish families, *Invest. Ophthalmol. Vis. Sci.*, 2002, vol. 43, no. 10, pp. 3160—3164.

72. Hameed, A., Khaliq, S., Ismail, M., et al., A novel locus for Leber congenital amaurosis (LCA4) with anterior keratoconus mapping to chromosome 17p13, *Invest. Ophthalmol. Vis. Sci.*, 2000, vol. 41, pp. 629—633.

73. Fullerton, J., Paprocki, P., Foote, S., et al., Identity-by-descent approach to gene localisation in eight individuals affected by keratoconus from north-west Tasmania, Australia, *Hum. Genet.*, 2002, vol. 110, pp. 462—470.

74. Bykhovskaya, Y., Li, X., Taylor, K.D., et al., Linkage analysis of high-density SNPs confirms keratoconus locus at 5q chromosomal region, *Ophthalmic Genet.*, 2014. doi 10.3109/13816810.2014.889172

75. Czugala, M., Karolak, J.A., Nowak, D.M., et al., Novel mutation and three other sequence variants segregating with phenotype at keratoconus 13q32 susceptibility locus, *Eur. J. Hum. Genet.*, 2012, vol. 20, no. 4, pp. 389—397. doi 10.1038/ejhg.2011.203

76. Kwofie, M.A. and Skowronski, J., Specific recognition of Rac2 and Cdc42 by DOCK2 and DOCK9 guanine nucleotide exchange factors, *J. Biol. Chem.*, 2008, vol. 283, no. 6, pp. 3088—3096. doi 10.1074/jbc.M705170200

77. Meola, N., Gennarino, V., and Banfi, S., microRNAs and genetic diseases, *PathoGenetics*, 2009, vol. 2, no. 1. doi 10.1186/1755-8417-2-7

78. Ambros, V., Bartel, B., Bartel, D.P., et al., A uniform system for microRNA annotation, *RNA*, 2003, vol. 9, pp. 277—279.

79. Jones, P.F., Jakubowicz, T., Pitossi, F.J., et al., Molecular cloning and identification of a serine/threonine protein kinase of the second-messenger subfamily, *Proc. Natl. Acad. Sci. U.S.A.*, 1991, vol. 88, pp. 4171—4175. doi 10.1073/pnas.88.10.4171

80. Hughes, A.E., Bradley, D.T., Campbell, M., et al., Mutation altering the miR-184 seed region causes familial keratoconus with cataract, *Am. J. Hum. Genet.*, 2011, vol. 89, no. 5, pp. 628—633. doi 10.1016/j.ajhg.2011.09.014

81. Lechner, J., Porter, L.F., Rice, A., et al., Enrichment of pathogenic alleles in the brittle cornea gene, ZNF469, in keratoconus, *Hum. Mol. Genet.*, 2014, vol. 23, no. 20, pp. 5527—5535. doi 10.1093/hmg/ddu253

82. Abu-Amero, K.K., Helwa, I., Al-Muammar, A., et al., Screening of the Seed Region of MIR184 in keratoconus patients from Saudi Arabia, *Biomed. Res. Int.*, 2015. doi 10.1155/2015/604508

83. Lu, Y., Dimasi, D.P., Hysi, P.G., et al., Common genetic variants near the Brittle Cornea Syndrome locus ZNF469 influence the blinding disease risk factor central corneal thickness, *PLoS Genet.*, 2010, vol. 6, no. 5. doi 10.1371/journal.pgen.1000947. e1000947

84. Vitart, V., Benčić, G., Hayward, C., et al., New loci associated with central cornea thickness include COL5A1, AKAP13 and AVGR8, *Hum. Mol. Genet.*, 2010, vol. 19, no. 21, pp. 4304—4311. doi 10.1093/hmg/ddq349

85. Vithana, E.N., Aung, T., Khor, C.C., et al., Collagen-related genes influence the glaucoma risk factor, central corneal thickness, *Hum. Mol. Genet.*, 2011, vol. 20, no. 4, pp. 649—658. doi 10.1093/hmg/ddq511

86. Lu, Y., Vitart, V., Burdon, K.P., et al., Genome-wide association analyses identify multiple loci associated with central corneal thickness and keratoconus, *Nat. Genet.*, 2013, vol. 45, no. 2, pp. 155—163. doi 10.1038/ng.2506

87. Gao, X., Gauderman, W.J., Liu, Y., et al., A genome-wide association study of central corneal thickness in Latinos, *Invest. Ophthalmol. Vis. Sci.*, 2013, vol. 54, no. 4, pp. 2435—2443. doi 10.1167/iovs.13-11692

88. Vincent, A.L., Jordan, C.A., Cadzow, M.J., et al., Mutations in the zinc finger protein gene, ZNF469, contribute to the pathogenesis of keratoconus, *Invest. Ophthalmol. Vis. Sci.*, 2014, vol. 55, no. 9, pp. 5629—5635.

89. Liu, Y., Abu-Amero, K.K., Bykhovskaya, Y., et al., Genomic deletions of RXRA-COL5A1, FAM46A-IBTK, HS3ST3B1-PMP22, and GRIA4 in familial keratoconus patients, *Proceedings of the ARVO Annual Meeting*, Orlando, Fla, 2014.

90. 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, vol. 21, no. 2, pp. 421—429. doi 10.1093/hmg/ddr460

91. Bae, H.A., Mills, R.D., Lindsay, R.G., et al., Replication and meta-analysis of candidate loci identified variation at RAB3GAP1 associated with keratoconus, *Invest. Ophthalmol. Vis. Sci.*, 2013, vol. 54, no. 7, pp. 5132—5135. doi 10.1167/iovs.13-12377

92. Aligianis, I.A., Johnson, C.A., Gissen, P., et al., Mutations of the catalytic subunit of RAB3GAP cause Warburg Micro syndrome, *Nat. Genet.*, 2005, vol. 37, no. 3, pp. 221—223. doi 10.1038/ng1517

93. Handley, M.T., Morris-Rosendahl, D.J., Brown, S., et al., Mutation spectrum in RAB3GAP1, RAB3GAP2, and RAB18 and genotype—phenotype correlations in Warburg Micro syndrome and Martsolf syndrome, *Hum. Mutat.*, 2013, vol. 34, no. 5, pp. 686—696. doi 10.1002/humu.22296

94. Morris-Rosendahl, D.J., Segel, R., Bor, A.P., et al., New RAB3GAP1 mutations in patients with Warburg Micro syndrome from different ethnic backgrounds and a possible founder effect in the Danish, *Eur. J. Human Genet.*, 2010, vol. 18, no. 10, pp. 1100—1106. doi 10.1038/ejhg.2010.79

95. Yüksel, A., Yesil, G., Aras, C., et al., Warburg Micro syndrome in a Turkish boy, *Clin. Dysmorphol.*, 2007, vol. 16, no. 2, pp. 89—93. doi 10.1097/mcd.0b013e328054c404

96. Burdon, K.P., Macgregor, S., Bykhovskaya, Y., et al., Association of polymorphisms in the hepatocyte growth factor gene promoter with keratoconus, *Invest. Ophthalmol. Vis. Sci.*, 2011, vol. 52, no. 11, pp. 8514—8519. doi 10.1167/iovs.11-8261

97. Sahebjada, S., Schache, M., and Richardson, A.J., Association of the hepatocyte growth factor gene with keratoconus in an Australian population, *PLoS One*, 2014, vol. 9, no. 1. doi 10.1371/journal.pone.0084067.e84067

98. Veerappan, S., Pertile, K.K., Islam, A., et al., Role of the hepatocyte growth factor gene in refractive error, *Ophthalmology*, 2010, vol. 117, no. 2, pp. 239—245. doi 10.1016/j.ophtha.2009.07.002

99. Yanovitch, T., Li, Y.-J., Metlapally, R., et al., Hepatocyte growth factor and myopia: genetic association analyses in a Caucasian population, *Mol. Vis.*, 2009, vol. 15, pp. 1028—1035.

100. Abu-Amero, K.K., Hellani, A.M., Al Mansouri, S.M., et al., High-resolution analysis of DNA copy number alterations in patients with isolated sporadic keratoconus, *Mol. Vis.*, 2011, vol. 17, pp. 822—866.

Translated by A. A. Kashevarova