The Genetic Background of Keratoconus: A Review on Keratoconus Genes

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Abstract

Keratoconus is a chronic, bilateral, usually asymmetrical, non-inflammatory, ectatic disorder, being characterized by progressive steepening, thinning and apical scarring of the cornea. It affects approximately 1 in every 2000 individuals, but its incidence seems to be increased with the clinical use of corneal topography. Keratoconus is considered as a multifactorial disease, caused by the interaction between several genes, microRNAs and environmental factors, including eye rubbing, atopy, sun exposure, geographic location and race. Although the disease is usually sporadic, a genetic predisposition and raised incidence in familial and monozygotic twins have been described. Given that the diagnosis of the disease is based on an anterior eye assessment, the identification of certain genes could be an additional diagnostic tool. Furthermore, it may pave the way for the gene therapy of the disease.

Keywords: Keratoconus; Diagnosis; Genes; Pathogenesis

Introduction

Keratoconus is a chronic, bilateral, usually asymmetrical, non-inflammatory, ectatic disorder, being characterized by progressive steepening, thinning and apical scarring of the cornea [1]. The prevalence of the disease had been estimated to be 1 in every 2000 individuals before the development of corneal topography devices, which raised the ratio to 54, 1190, 2300, 3.3 and 20 per 100,000 in USA, France, India, Iran (Tehran) and Middle East, respectively [1-3]. Structural abnormalities in the corneal epithelium, Bowman’s layer and stroma along with altered concentration of tear components are responsible for its clinical features [1,2]. The progressive myopia and the irregular astigmatism, are implicated in the decreased resolution observed in keratoconus [1,2]. Changes in aberrations and haloes around objects or lights finally result in poor quality of vision and life [1,2]. The keratometric, retinoscopic or slit lamp findings put the diagnosis of clinical keratoconus, while the subclinical type exhibits only mild topographic changes [1].

Clinical features and diagnosis

Keratoconus is a bilateral, progressive condition which is usually stabilized by the fourth decade of life in a significant loss of vision and patients’ quality of life [1,2,4,5]. In early stages, the patient is asymptomatic, but subsequently the visual acuity gradually decreases [4]. The progressive corneal thinning associated with keratoconus [mean central corneal thickness (CCT) 450-500 μm] results in myopia, irregular corneal astigmatism and finally in a significant loss of vision [4]. Rigid gas-permeable contact lenses have been found to rehabilitate sufficiently the vision, resulting in a higher quality of life, compared to keratoconic spectacles wearers [6]. In healthy humans, CCT is a normally distributed quantitative trait with a mean of 536 ± 31 μm, which has an estimated heritability up to 95% [7,8]. The early biomicroscopic signs of keratoconus include the appearance of Fleischer’s ring, which is a partial or complete circle of iron deposition in the epithelium surrounding the base of the cornea and Vogt’s striae [9]. The latter consists of fine vertical lines produced by the compression of Descemet’s membrane [9]. An oil droplet reflex can be seen using direct ophthalmoscopy, whereas retinoscopy reveals an irregular scissor reflex [9]. As the disease progresses, a Munson’s sign appears, being characterized by a V-shaped deformation of the lower lid, when the patient looks downwards [9]. Rizzuti’s sign represents a bright reflection of the nasal area of the limbus, being also related to advanced disease [10]. Corneal scarring is a common sign of contact lenses wearing [11]. Contrariwise, breaks in Descemet's membrane appear less frequently, leading to hydrops, which is described by stromal edema, vision loss, and associated pain [9].

The basic diagnostic examinations for keratoconus include placido disk–based corneal topography, Orbscan I (Bausch & Lomb, Rochester, New York, USA) and II slit topography, Pentacam (Oculus, Wetzlar, Germany) Scheimpflug imaging, wavefront aberrometers and spectral domain optical coherence tomography (SD-OCT) [12]. On the other hand, confocal microscopy, Oculus Response Analyzer (ORA, Reichert Inc., Depew, New York, USA) and Fourier Transform Infrared (FTIR) spectroscopy are relatively recent diagnostic tools [12].

Pathogenesis of keratoconus

Biomechanics, enzymes, proteomics, and molecular genetics are implicated in the pathogenesis of keratoconus. Altered expression of extracellular matrix proteoglycans, such as decorin, lumican, biglycan and keratanoc, and proteins, along with decreased stromal collagen content are the basic structural changes observed in keratoconus. The changes in extracellular components result in distortion of collagen fibers and lamellae, eliminating corneal strength and transparency [13,14]. Besides the alterations of the collagen, the disturbances of cell junctions have been also associated with decreased levels of transforming growth factor beta (TGF-β) [14]. The TGF-β1 and/or TGF-β3 isoforms seem to regulate mitochondrial proteins in human
corneal fibroblasts, being implicated in the pathogenesis of keratoconus [15]. The accumulation of proteolytic enzymes, including cathepsin-B, -G, -V/L2 and lysosomal enzymes are implicated in the degradation of the collagen and the cell death observed in keratoconus [14]. Furthermore, cathepsins regulate cell apoptosis and mitochondria function, contributing to oxidative stress [14].

Disturbances in lipid peroxidation and nitric oxide pathways may lead to accumulation of toxic products along with apoptosis of the corneal cells [16]. The loss of β-actin or high levels of cytokines, including interleukin 1 and 6, favors the cellular apoptosis [16]. The increased susceptibility of keratoconic corneas to injury has been also related to changes in the expression of genes associated with wound healing, including the nerve growth factor and the visual system homeobox 1 (VXV) [16]. Increased mtDNA content in patients with keratoconus may indicate mitochondrial respiratory chain defects [17]. An imbalance between matrix metalloproteinases-2 (MMPs-2) and their tissue inhibitors (TIMPs), which exhibit anti-apoptotic properties, has been also related to corneal thinning [18]. The expression of MMPs and cell apoptosis are impaired by the high levels of interleukins, which are released by the eye rubbing and chronic contact lenses wearing. High levels of TGF-β1 and INPPL-1 (inositol polyphosphate-5 phosphatase-like 1) messenger ribonucleic acid (mRNA) have been also measured in eyes with keratoconus [18].

Environmental factors, including eye rubbing, atopy, and sun (Ultra Violet radiation) exposure have been implicated in the pathogenesis of keratoconus [19]. In some reports, coexistence of KTCN with atopy and allergy was presented [20-22]. Moreover, a positive familial history and the parental education and socioeconomic status seem to impair the development of the disease [19]. Keratoconus has been also estimated to exhibit different distribution, depending on geographic location and race [19]. The incidence of the disease is 4.4 times higher in Asians (Indians, Bangladesh, and Pakistani) living in the English Midlands in whites [19]. Moreover, the prevalence of keratoconus is low in Northern Europe, Japan, Urals and USA, in contrast with the one observed in Middle East, India and China [19]. Finally, the age seems to affect the development of keratoconus; the mean diagnostic age ranges from 20.0 to 24.05 years, whereas it seldom appears after the age of 35 years [19]. Furthermore, keratoconus has been related to systemic and ocular conditions, including Leber congenital amaurosis, anterior polar cataract, Down syndrome (10–300-fold higher prevalence) and Ehlers Danlos syndrome [13].

### Purpose-methods

This is literature review of several important articles focusing on the genetic background of keratoconus. Relevant publications in the PUBMED database were searched for articles regarding the genes which have been implicated in the pathogenesis and progression of the disease.

### The Genetic Background of Keratoconus

Keratoconus is considered as a multifactorial disease, caused by the interaction between several genes and environmental factors. Although the disease is usually sporadic, a genetic predisposition and raised incidence in familial and monoyzygotic twins have been described [13,18]. The modes of keratoconus inheritance are usually dominant and recessive, but incomplete penetrance with variable phenotype appears in the autosomal dominant inheritance [19]. Kriszt et al. suggested that KISA (Keratoconus percentage index), KIS (Keratoconus Severity Index) and Fourier 6 asymmetry indices are probably inherited by a non-mendelian major gene effect [22].

### The role of miRNAs and RNA in the pathogenesis of keratoconus

MicroRNAs (miRNAs) are able to recognize their target mRNAs by using as little as 6–8 nucleotides (the seed region) at the 5’ end of the miRNA, which is not enough pairing to induce cleavage of the target mRNAs. A given miRNA may have hundreds of different mRNA targets, and a given target might be regulated by multiple miRNAs. Recently, Moschos et al. observed a high incidence of hsa-mir-568 (human serum albumin miRNA 568) rs149509568 polymorphism in Greek patients with sporadic keratoconus (P=0.04, odds ratio (OR): 5.08, 95% confidence interval: 0.97-26.61), suggesting a potential role of the has-mir-568 in the pathogenesis of the disease [23]. No significant association was detected between the rs41280052 (located within the pre-miR-184 sequence) polymorphism and keratoconus. Indeed, they noted that the T allele of the rs41280052 was present in 5.74% of KC patients and in 8.75% of healthy controls [P=1.00, OR: 1.82, 95% confidence interval: 0.11-29.66] [23]. MiR-184 has been found to be expressed in central basal and suprabasal epithelial cells, under which the stromal thinning occurs in keratoconus [24]. The harmful effects of mutant miR-184 might be mediated through INPPL-1 (inositol polyphosphate-5 phosphatase-like 1) and ITGB4 ( Integrin, beta 4), which is a transmembrane glycoprotein receptor [24]. This mutation in the seed region of miR-184 (MIR184) has been implicated in familial severe keratoconus combined with early-onset anterior pectoral cataract [24].

### Genes implicated in oxidative stress

Giving that oxidative stress is implicated in the pathogenesis of keratoconus, Synowiec et al. investigated the c.977C>G polymorphism of the hOGG1 (8-Oxoguanine glycosylase) gene (rs1052133) and the c. 972G>C polymorphism of the MUTHY (mutY DNA glycosylase) gene (rs3219489) [25]. The products of both hOGG1 and MUTHY genes contributed notably in the repair of oxidatively modified DNA in the base excision repair pathway [25]. However, these polymorphisms were not related to keratoconus occurrence in Polish population [25].

The base excision repair (BER) which is performed in corneal biomolecules after any oxidative stress damage, has been suggested to be responsible for the pathogenesis of keratoconus [26]. Polymorphisms of X-ray repair cross-complementing group 1 (XRCC1) and polymerase gamma (POLG) genes have been also related to keratoconus [26]. The A/A genotype of the c.-1370T>A polymorphism of the DNA POLG gene was associated with increased incidence of keratoconus, while the A/T genotype was related to reduced occurrence of keratoconus [26]. The A/G genotype and the A allele of the c.1196A>G polymorphism of the XRCC1 were estimated to favor keratoconus, whereas the G/G genotype and the G allele, were found to diminish the development of keratoconus [26]. Furthermore, the C/T and T as well as the C/C genotypes and alleles of the c.580C>T polymorphism of the same gene were correlated with keratoconus [26]. Mitochondrial respiratory chain defects have been also involved in the failure of keratoconic corneas to respond to the oxidative stress [26].

Given that iron may promote the Fenton reaction, leading to the production of highly reactive hydroxyl radicals (OH·), the correlation of three polymorphisms [g.3296G>A (rs8177178), g.3481A>G (rs8177179), and c.-2G>A (rs1130459)] of the transferrin gene was...
investigated [27]. Reactive oxygen species (ROS), including OH\(^-\), can lead to oxidative stress, modifying the structure and functions of the cellular components [27]. The A/A genotype and the A allele of the g.3296G>A polymorphism were associated with the prevalence of keratoconus, while the G allele was negatively correlated with it [27]. The A/G genotype of the g.3481A>G polymorphism exhibited protective action against keratoconus. No association between the c.–2G>A polymorphism and keratoconus was detected [27].

Pathak et al. investigated mitochondrial complex I genes (ND1, 2, 3, 4, 4L, 5, and 6), related to oxidative stress [28]. The majority of the mutations were found in ND5 (n=28) followed by ND4 (15) and then ND2 [28]. Most of the patients and their maternal relatives were clustered under the haplogroups (T, C4a2a, R2*TJ, M21*Q1a, M12*G2a2a, M8*CZ, M7a2a, U5b1, U1a3), which were present as negligible frequency in normal Indian population [28]. Only few patients were found to be a part of the haplogroups, whose origin is contentious, i.e. U7 (Indo-European), R2 and R31 [28]. The mutations which were observed in patients with keratoconus can affect transcription, translation or have synergistic effect with other variants in causing the disease [28]. Pathak et al. concluded that sequence variation in mitochondrial complex I gene in keratoconus patients are associated with depleted or low ATP levels, raised ROS and malondialdehyde (MDA) levels which can lead to altered protein function, apoptosis and damage of corneal tissues [28].

Disturbances in genes responsible for inflammation, apoptosis cellular growth and differentiation

Disturbances in inflammation and in cellular growth, differentiation, and motility, following the mutations of interleukin-1 (IL1), may be responsible for the corneal changes observed in keratoconus [29]. Altered levels of IL1 may be associated with keratoconus, assessing three single-nucleotide polymorphisms (SNPs) (rs2071376 in IL1A, rs1143627 and rs16944 in the promoter region of IL1B) in Chinese Han patients [29]. The A allele of rs2071376 (A>C, p=0.017, OR=1.968, 95% CI 1.313-3.425), the C allele of rs1143627 (C>T, p<0.001, OR=2.864, 95% CI 1.631-4.968) and the A allele of rs16944 (A>G, p=0.002, OR=2.401, 95% CI 1.396-4.161) were considered to promote keratoconus [29]. Keratocyte apoptosis is an initiating event in the pathogenesis of KC which could be induced by the altered levels of IL1 gene [29].

The rs1143627 (–31 T>C) SNP in IL1B promoter region was related to keratoconus in a Japanese population; the T allele of rs1143627 was found to significantly increase the risk of keratoconus (OR=1.38) [30]. In the same study, no significant differences were found in the allele and genotype frequencies between the cases and controls for rs2071376 in IL1A [30]. The c.2558+149_2558+203del54 in SLC4A11 (sodium bicarbonate transporterlike protein 11) and c.214+242C>T in IL1RN (gene encoding IL-1ra protein) sequence variants have been also implicated in the pathogenesis of the disease [31]. Wang et al. also revealed that rs2071376 in the IL1A gene raised the risk for keratoconus (OR=1.51) [32]. They further noted that three tSNPs and three haplotypes in the V5X1 gene were over-presented in keratoconic patients [32].

Keratoconic corneas have been also associated with higher level of DUSP1 and TGF-β1 expression [18]. DUSPs regulate responses in positive and negative ways and are key regulators of immune responses [18]. TGF-β1, which is associated with various corneal dystrophies, is involved in regulating keratocyte activation, myofibroblast transformation and proliferation, chemotaxis, and wound healing [18]. Guan et al. also detected genetic variations and mutations of TGFβ1 gene in keratoconus among Chinese population [33].

Nowak et al. analyzed known keratoconus loci to uncover genetic factors involved in this disease causation in the general population [34]. They revealed 1,045,902 Single Nucleotide Variations (SNVs) in 1,000 Genome database located within over two thousands of various genes [34]. Subsequently, for 289 genes, in which these SNVs were located, the ranking based on topological features in protein-protein interaction network was created [34]. From all tested genes, SULF1, CCDC80, FARPI, PDGFRB, and VCAN got the highest five ranks [34]. Protein encoded by FARPI gene regulates dendritic filopodial dynamics in immature neurons and contributes to synapse formation [34]. CCDC80 (Coiled-coil domain-containing protein 80) encodes a protein involved in the induction of C/EBPβs (C/EBPα-enhancer-binding proteins, family of transcription factors) and peroxisome proliferator-activated receptor γ (PPARY) [34]. The latter acts as a negative regulator in immune cells, suppresses the expression of thymic stromal lymphopoietin in the skin, and suspends the maturation of dendritic cells in a mouse model of atopic dermatitis [34]. Both SULF1 (Sulfatase 1) and PDGFRB (Beta-type platelet-derived growth factor receptor) take part in corneal wound healing [34]. VCAN gene encodes a versican, which is an extracellular matrix protein and component of the vitreous, being implicating in its structural integrity [34]. The mutation of the VCAN gene has been related to Wagner syndrome [34]. For further analysis, genes predicted as targets for miRNAs from keratoconus loci were ranked based on topological features in protein-protein interaction network [34]. The three highest ranked genes were SMAD2, CAND1 (Gullin-associated NEDD8-disassociated protein 1), and VHL (von Hippel–Lindau tumor suppressor). SMAD2 protein mediates the signal of TGF-β, regulating multiple cellular pathways [34].

The Apoptosis Stimulating Fragment (FAS) protein is a cell-surface receptor, belonging to tumour necrosis factor (TNF) receptor superfamily [26]. It may induce apoptosis upon its ligand (FASLG) binding and this pathway is as a primary mechanism for the induction of apoptosis in many types of cells and tissues, including eye, testis and maternal-foetal interface [26]. The c.–671A>G polymorphism of the apoptosis-related FAS gene and the c.–844T>C polymorphism of the FASLG gene were investigated in patients with keratoconus [26]. The T/T genotype and the T allele of the c.–844T>C polymorphism were associated with increased occurrence of keratoconus, while the C allele was associated with decreased keratoconus occurrence [26]. Although no correlations between genotypes/alleles of the c.–671A>G polymorphism and the occurrence of keratoconus were detected, the T/T/G/A combined genotype was related to high incidence of the disease [26].

Polymorphisms in genes encoding collagen and cellular cytoskeleton proteins

Reduced amounts of total collagen proteins and collagen type IV have been implicated in the pathogenesis of keratoconus. The possible associations between collagen type IV alpha-4 chain (COL4A4) polymorphisms (rs2229813 G/A, M1327Y and rs2228555 A/G; V1516V) and susceptibility to keratoconus were estimated by Saravani et al. [35]. The COL4A4 rs2229813 AA and GA+AA genotypes were considered as risk factors for developing keratoconus (OR=2.1, P=0.036 and OR=1.7, P=0.042, for the AA and GA+AA genotypes, respectively). The COL4A4 rs2229813 A allele was also associated with
an increased risk for keratoconus (OR=1.5, 95% confidence intervals: 1.1-2.2, P=0.018) [35]. In Greek population the mutations in COL4A3 and COL4A4 genes were not associated with risk for keratoconus [36]. Contrariwise, the M1327V AA and F1644F TT alterations in proteins levels were related to atherosclerosis signaling, receptors acting as transcription factors) and granzyme A signaling.

Besides collagen IV, the transcript levels of collagen I and Lysyl oxidase genes have been found to be diminished in keratoconic patients [37]. Lysyl oxidase is a copper-dependent amine oxidase, which oxidizes the epsilon amino groups of peptide lysines into reactive aldehydes [37]. Thus it is responsible for the development of lysine-derived crosslinks in extracellular matrix proteins, such as collagen and elastin [37].

The transcript of the RNA expression levels of these three corneal structure-related genes has been associated with the severity of keratoconus [37]. Furthermore, the coexistent increase in the levels of MMP9 suggests a possible regulatory signaling loop between these collagen-degrading and cross-linking enzymes, being involved in the pathogenesis of keratoconus [37].

Chaerkady et al. investigated the epithelial and stromal proteome from normal donor and keratoconic corneas [38]. A total of 932 and 1,157 proteins were detected in the consolidated data of the epithelium and stroma, respectively [38]. They discovered that some proteins were increased in keratoconic corneas, including type I cytotkeratin KRT16 (5-fold), type II cytotkeratin KRT6A (5-fold), vimentin (2-fold), Hemoglobin beta, Cysteine-rich protein 1 and Calmodulin-like 3 [38]. Contrariwise, basement membrane and sub-epithelial collagen types VII and XII (COL7A1, COL12A1), all three chains of collagen type VI (COL6A1, COL6A2 and COL6A3) and iron transporter Lactotransferrin were found to be reduced in keratoconus [38]. These alterations in proteins levels were related to atherosclerosis signaling, liver X receptor (LXR)/retinoid X receptor (RXR) activation (nuclear receptors acting as transcription factors) and granzyme A signaling [38]. The latter leads to hydrolysis of collagen type IV [38]. Moreover, nuclear factor (erythroid-derived 2)-like 2-mediated oxidative stress and mitochondrial dysfunction are noted. The elongation initiation factor subunit (EIF2S2), MME (matrix metalloepopeptidase), Ca-binding RCN1 (Reticulocalbin-1), and CSNK2B (Casein kinase II subunit beta) that localize to the endoplasmic reticulum and Golgi to regulate translation were decreased in the stroma of keratoconic corneas [38].

Collagen types VI and XII were also diminished in the stroma along with nestin and crystallins beta B1, beta B2 and gamma S, which are responsible for lens transparency [38]. On the other hand, several 40S and 60S ribosomal proteins were all raised indicating accumulations of ribosomal proteins and endoplasmic reticulum stress [38]. These proteins involved the Adaptor-related Protein complex 1 and 2 (beta 1 subunit), Immunoglobulin Lambda-like polypeptide 1 and Cell division cycle and apoptosis regulator 1, which regulates cellular apoptosis [38]. The reported reduction in hydroxylated peptides could also relate to the general oxidative stress and endoplasmic reticulum dysfunction [38]. Chaerkady et al. concluded that keratoconic stromal proteome included a broad decrease in many structural collagens and proteoglycans, a minor increase in proteases, altered apoptosis related proteins and complement components that suggest abnormal lipid metabolism, complement functions and cell death as major keratoconic processes [38].

Macé et al. suggested that anti-proliferative and hyperapoptotic phenotypes may be responsible for the pathogenesis of keratoconus [39]. They investigated the RNA of 10 keratoconic corneas and identified 87 genes, including 69 downregulated (e.g FOS, JUN, FOSB, MYC, and C2CNIA) and 18 overexpressed genes [39]. The latter encoded for proteins being involved in the extracellular matrix and the epithelial cell cytoskeleton (PTCH2, KRT5, KRT78, and LYPD3), in the stress response (HSP90AA1 and ALDH1A3), or mucins (MUC4 and MUC16) [39]. An older study of Karolak et al. supported that keratoconus was not related with mutations in COL4A1 and COL4A2 [40]. On the other hand, they determined three missense substitutions in COL4A1, including c.19G>C (Val7Leu), c.1663A>C (Thr555Pro), and c.4002A>C (Gln1334His) [40]. Furthermore, five non-synonymous variants were identified in COL4A2 c.574G>T (Val192Phe), c.1550G>A (Arg517Lys), c.2048G>C (Gly683Ala), c.2102A>G (Lys701Arg), and c.2152C>T (Pro718Ser) [40].

Genes related to corneal curvature and thickness

Han et al. identified two genes in Asian populations that can influence corneal curvature and possible keratoconus development; FRAPI on chromosome 1p36.2 and PDGFRA on chromosome 4q12 [41]. FRAPI encodes for FK506 binding protein 12-rapamycin associated protein 1, which binds to the phosphatidylinositol 3-kinase-related (PI3 kinase) family protein, and regulates cellular growth and proliferation, as well as transcription [41]. PDGFRA encodes for the alpha-isofrom of the Platelet-derived growth factor receptors (PDGF-R), which are catalytic receptors with intracellular tyrosine kinase activity [41]. The kinase activity of both proteins explains their effect on corneal epithelial cells and collagen fibrils and subsequently on corneal curvature [41]. A recent study confirms the implication of PDGFRA in corneal curvature, detecting SNPs of PDGFRA and TRIM29 in Australians of Northern European ancestry [42]. Tripartite motif-containing protein 29 (TRIM29) with multiple zinc finger motifs and a leucine zipper motif, acts as a transcriptional regulatory factor and is involved in carcinogenesis and/or differentiation [42]. However, the same study did not reveal any effect of FRAPI on corneal curvature [42].

IBTK on chromosome 6q14.1, CHSY1 on chromosome 15q26.3, and intergenic regions on chromosomes 7q11.2 and 9p23 in Chinese population were found to influence central corneal thickness [43]. IBTK is the inhibitor of Bruton’s tyrosine kinase (BTK), which downregulates BTK kinase activity, BTK-mediated calcium mobilization and the activation of nuclear factor-kappa-B(NF-xB)- driven transcription [43]. It has been suggested that IBTK modulates corneal development through its negative regulation of BTK activity and that this occurs via the Wnt-β-catenin signalling pathway [43]. The enzyme encoded by chondroitin sulphate synthase 1 (CHST7) gene synthesizes chondroitin sulphate, a glycosaminoglycan observed abundantly in the corneal stromal extracellular matrix [43]. Alterations in these regions could lead to keratoconic features [43].

Genes associated with systemic diseases and syndromes

Pathogenic alleles in ZNF469 (zinc-finger protein 469) gene have been also estimated as genetic factor responsible for keratoconus [44]. The encoding protein is a transcription factor being involved in the synthesis and organization of collagen fibers, whereas mutations in these genes have been associated with Type 1 brittle cornea syndrome (BCS) [44]. The latter is an autosomal recessive generalized connective tissue disorder associated with severe progressive corneal thinning (220–450 µm) and cataract, high myopia, blue sclera and predisposition to corneal rupture [44]. Although PRDM5 gene mutations have been implicated in the pathogenesis of Type 1 BCS, Lechner et al. did not...
detect any pathogenic variants [44]. However, Rohrbach et al. investigated both these genes and identified a single patient who did not have a mutation in either ZNF469 or PRDM5, suggesting genetic heterogeneity in BCS [45]. An older study suggested the hypothesis that PRDM5 and ZNF469 regulate extracellular matrix organization through similar biochemical mechanisms [46]. Fibroblasts from BCS patients with both PRDM5 and ZNF469 mutations were investigated and revealed similar cellular phenotypes, with disruption in the deposition of several collagenas, fibronectin and integrins [46]. Microtripllication 11q24.1 has been associated with facial dysmorphisms, short stature with small extremities, keratoconus, overweight, and intellectual disability [47].

Two genes encoding for proteins that interact with Tuberous Sclerosis Complex 1 (TSC1) and 2 (TSC2) have been also identified in keratoconic corneas: ribosomal protein S6 kinase 70-kDa (RPS6KB1) and FKB12-rapamycin complex-associated protein 1 (FRAP1) [46]. Keratoconus has been also detected in patients with Williams-Beuren syndrome [48]. The latter is a genetic multisystemic neurodevelopmental disorder caused by a contiguous gene deletion at 7q11.23 [48]. Mutations of filaggrin in keratoconic patients indicate a possible common aetiology with ichthyosis vulgaris and atopic dermatitis [49].

Other genes implicated in keratoconus pathogenesis

Karolak et al. detected sequence variants of VSX1, TGF-β1, DOCK9, IPO5, and STK24 genes in a small proportion of Polish keratoconic patients [50]. VSX1 regulates the expression of the cone opsin genes early in development, while mutations in this gene can cause posterior polymorphous corneal dystrophy [50]. DOCK9 (Dedicator of cytokinesis) is involved in intracellular signaling networks, while STK24 (Serine/threonine-protein kinase) functions upstream of mitogen-activated protein kinase (MAPK) signaling [50]. IPO5 (Importin-5) is a type of karyopherin, binding protein molecules into the nucleus by binding them to recognition sequences, called nuclear localization sequences (NLS) [50]. Variants c.-264_+255delGGGGTTGTTGGGT, +627 +23G>A, c.809-6_809-5insT and c.*200G>T in the VSX1 gene, and heterozygous c.1598G>A (Mutation Arg533Gln) in the 12 of TGFBR1 were detected for the first time in keratoconic patients [50]. The H244R mutation in exon 4 of VSX1 has also been identified in keratoconic patients in southwest Iran [51].

On the other hand Moschos et al. reported no polymorphisms of VSXI gene related to keratoconus [52]. However, in the same study the SOD1 intronic 7-base deletion (c.169+50delTAAACAG) was over-represented among keratoconic patients compared to healthy controls [52]. R166W and H244R VSXI variants might play critical role in the pathogenesis of keratoconic corneas, as suggested by Saeed-Rad et al. [53]. The same study group identified three novel SNPs (g.4886G>A, g.4990C>G, and g.9061T>A) in SOD1, but these SNPs did not seem to influence the activity of SOD1 protein [53]. Cruzaga et al. detected four substitutions in three different genes: c.2262A>C (p.Gln754His) and c.720+43A>G in DOCK9, c.2577-132A>C in IPO5 and c.1053+29G>C in Serine/threonine-protein kinase (STK24) [54]. Although they associated all variants with keratoconus, only c.2262A>C (p.Gln754His) mutation in DOCK9 was exclusively observed in keratoconic phenotype [54].

The mutations p.L17P, p.N151S, p.G160V, p.R166W, p.Q175H, and p.G239R of VSXI gene were exclusively designated in Italian keratoconos patients by De Bonis et al. [55]. Furthermore, the c.169+50delTAAACAG deletion of SOD1 gene was detected in two sporadic cases of keratoconus by the same study group [55]. Sequencing analysis of the SPARC (Secreted Protein Acidic and Rich in Cysteine) gene also revealed three novel variants leading to the amino acids substitutions p.E63K, p.M921, and p.D219E [55]. SPARC is a glycoprotein secreted by osteoblasts during bone formation. It favors mineral crystal formation and exhibits high affinity for collagen in addition to bone mineral calcium [55]. Analysis of Lysyl Oxidase (LOX), and Tissue Inhibitor of Metalloproteinase 3 (TIMP3) genes excluded their possible involvement in the pathogenesis of keratoconus [55].

**Conclusions**

Keratoconus is a chronic, bilateral, usually asymmetrical, non-inflammatory, ectatic disorder, being characterized by the progressive steepening, thinning and apical scarring of the cornea. It affects approximately 1 in every 2000 individuals, but its prevalence seems to be increased due to the development of corneal topography devices. Patients with keratoconus are initially asymptomatic, but myopia, irregular corneal astigmatism and a significant loss of vision are finally established as the corneal thinning progresses.

Keratoconus is considered as a multifactorial disease, caused by the interaction between several genes, microRNAs and environmental factors, including eye rubbing, atopy, sun exposure, geographic location and race. Although the disease is usually sporadic, a genetic predisposition and raised incidence in familial and monozygotic twins have been described. The inefficient of keratoconic corneas to respond to the oxidative stress and mitochondrial respiratory chain defects have been associated with polymorphisms of hOGG1, XRCCI, POLG, transferritin and mitochondrial complex I genes. Changes in the levels of inflammatory mediators, such as IL1 and TGF-β1 or disturbances in apoptosis participate in the tissue damage observed in keratoconus. Altered expression of extracellular matrix components and collagen alterations with disturbed wound healing explain the clinical and histological features of keratoconic corneas. Type I cytokeratin KRT16 and type II cytokeratin KRT6A, KRT5, KRT7, vimentin, Cysteine-rich protein 1 and Calmodulin-like 3, FOS, JUN, FOSB, PTCH2, LYPD3, ALDH1A3 and mucus, COL1A1, COL2A1, COL4A3, COL4A4, COL7A1, COL12A1, and all three chains of collagen type VI are genes susceptible to modifications related to the abovementioned features. SNPs of FRAP1, PDGFRα or CHST1 seem to affect corneal curvature and thickness. Systemic disorders, including brittle cornea syndrome Type 1 syndrome, Tuberous Sclerosis Complex and Williams-Beuren syndrome can also be accompanied with keratoconus.
Given that the diagnosis of the disease is based on a combination of ophthalmological examinations, none of which is specific for keratoconus, the identification of certain genes could be an additional diagnostic tool. Furthermore, the genetic basis of keratoconus provides an opportunity to apply molecular studies to determine the mechanisms responsible for the development of keratoconus, whereas it may pave the way for personalized treatments of the disease.

Commentary

The authors believe that increasing our knowledge on keratoconus genetics and epigenetics will take us one step further in personalized medicine. The challenge is to have better phenotype/genotype correlation since there are a number of disease trait phenotypes. The functional role of the SNPs indentified should be clarified. Multi-omics study (genetics, epigenetics, metabolomics and proteomics) in well-defined phenotypes will offer new opportunity to dissect the genetic background of keratoconus.

Declaration of Interest

All authors have no conflict of interest to declare and no financial support was offered for the present review. The authors alone are responsible for the content and writing of this paper.

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