Journal of Clinical & Experimental Ophthalmology

Moschos et al., J Clin Exp Ophthalmol 2016, 7:6
DOI: 10.4172/2155-9570.1000617

Review Open Access

The Genetic Background of Keratoconus: A Review on Keratoconus Genes

Marilita M Moschos[1*], **Maria Gazouli**[2] **and Eirini Nitoda**[1]

[1]*First Department of Ophthalmology, Medical School, National & Kapodistrian University of Athens, Greece*

[2]*Department of Basic Medical Science, Laboratory of Biology, Medical School, National & Kapodistrian University of Athens, Greece*

[*]**Corresponding author:** Marilita M Moschos, Associate Professor, First Department of Ophthalmology, Medical School, National & Kapodistrian University of Athens, 6, Ikarias street, Ekali, 14578, Athens, Greece, Tel: +306944887319; Fax: +302104122319; E-mail: moschosmarilita@yahoo.fr

Received date: October 24, 2016; **Accepted date:** December 06, 2016; **Published date:** December 12, 2016

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 a 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 ophthalmopscopy, 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 tomogaphy (SD-OCT) [12]. On the other hand, confocal microscopy, Ocular 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 keratocan, 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 (*VSX1*) [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 dual-specificity phosphatase 1 (DUSP1) 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, Bangladeshi, 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 monozygotic 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 polar 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 *MUTYH* (mutY DNA glycosylase) gene (rs3219489) [25]. The products of both *hOGG1* and *MUTYH* 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 ($OH^{\bullet}$), 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 $OH^{\bullet}$, 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 have been 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% C.I. 1.313-3.425), the C allele of rs1143627 (C>T, p<0.001, OR=2.864, 95% C.I. 1.631-4.968) and the A allele of rs16944 (A>G, p=0.002, OR=2.401, 95% C.I. 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 *VSX1* 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 *TGFB1* 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*, *FARP1*, *PDGFRB*, and *VCAN* got the highest five ranks [34]. Protein encoded by *FARP1* 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/EBPα (CCAAT-enhancer-binding proteins, family of transcription factors) and peroxisome proliferator-activated receptor γ (*PPARγ*) [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 (Cullin-associated NEDD8-dissociated 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, M1327V 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 genotypes were significantly over-represented in healthy individuals, suggesting a protective role of these genotypes against disease [36].

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 peptidyl 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 reduction of the mRNA 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 cytokeratin KRT16 (5-fold), type II cytokeratin 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 metalloendopeptidase), 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 *CDKN1A*) 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; *FRAP1* on chromosome 1p36.2 and *PDGFRA* on chromosome 4q12 [41]. *FRAP1* encodes for FK506 binding protein 12-rapamycin associated protein 1, which belongs to the phosphatidylinositol 3-kinase-related (PI3 kinase) protein family, and regulates cellular growth and proliferation, as well as transcription [41]. *PDGFRA* encodes for the alpha-isoform 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 *FRAP1* 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-κB)-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 (*CHSY1*) 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 ectasia, 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 collagens, fibronectin and integrins [46]. Microtriplication 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 FKBP12-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 neuro-developmental 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, transporting protein molecules into the nucleus by binding them to specific recognition sequences, called nuclear localization sequences (NLS) [50]. Variants c.-264_-255delGGGGTGGGGT, c.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 exon 12 of *TGFBI* were detected for the first time in keratoconic patients [50]. The H244R mutation in exon 4 of *VSX1* has been also identified in keratoconic patients in southwest Iran [51].

On the other hand Moschos et al. reported no polymorphisms of *VSX1* 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 VSX1 variants might play critical role in the pathogenesis of keratokonic corneas, as suggested by Saee-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]. Czugala et al. detected four substitutions in three different genes: c.2262A>C (p.Gln754His) and c.720+43A>G in *DOCK9*; c.2377-132A>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 *VSX1* gene were exclusively designated in Italian keratoconus 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.M92I, 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].

MPDZ-NF1B gene encodes a protein containing multiple PDZ domains and promoting the binding of HTR2C (a subtype of 5-HT receptor that binds the endogenous neurotransmitter serotonin) receptor at cellular surfaces [16]. The protein encoded by BANP is a a tumor suppressor and cell cycle regulator [16]. The tag SNP (tSNP) rs2286194 of hepatocyte growth factor (HGF) has been also involved in keratoconus development [16]. Sahebjada et al. associated keratoconus with the SNPs rs1324183 in the *MPDZ-NF1B* gene and rs9938149 (between BANP and the zinc-finger protein ZNF4659) [56]. Moreover, Burdon et al. correlated the SNP rs3735520 of the *HGF* gene not only with keratoconus but also with serum HGF levels in control participants [57].

Conclusions

Keratoconus is a chronic, bilateral, usuallly 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 response to the oxidative stress and mitochondrial respiratory chain defects have been associated with polymorphisms of *hOGG1*, *XRCC1*, *POLG*, transferrin 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 along with disturbed wound healing explain the clinical and histological features of keratoconic corneas. Type I cytokeratin *KRT16* and type II cytokeratin *KRT6A*, *KRT5*, *KRT78*, vimentin, Cysteine-rich protein 1 and Calmodulin-like 3, *FOS*, J*UN*, *FOSB*, *PTCH2*, *LYPD3*, *ALDH1A3* and mucins, *COL4A1*, *COL4A2*, *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*, *PDGFRA* or *CHSY1* seem to affect corneal curvature and thickness. Systemic disorders, including brittle cornea syndrome Type 1 syndrome, Tuberous Sclerosis Complex and Williams-Beuren syndrome can be also 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.

References

1. Serdarogullari H, Tetikoglu M, Karahan H, Altin F, Elcioglu M (2013) Prevalence of keratoconus and subclinical keratoconus in subjects with astigmatism using pentacam derived parameters. J Ophthalmic Vis Res 8: 213-219.
2. Hashemi H, Khabazkhoob M, Yazdani N, Ostadimoghaddam H, Norouzirad R, et al. (2014) The prevalence of keratoconus in a young population in Mashhad, Iran. Ophthalmic Physiol Opt 34: 519-527.
3. Bialasiewicz A, Edward DP (2013) Corneal ectasias: study cohorts and epidemiology. Middle East Afr J Ophthalmol 20: 3-4.
4. Gordon-Shaag A, Millodot M, Kaiserman I, Sela T, Barnett Itzhaki G, et al. (2015) Risk factors for keratoconus in Israel: a case-control study. Ophthalmic Physiol Opt 35: 673-681.
5. Gomes JA, Tan D, Rapuano CJ, Belin MW, Ambrósio R Jr, et al. (2015) Global consensus on keratoconus and ectatic diseases. Cornea 34: 359-369.
6. Ortiz-Toquero S, Perez S, Rodriguez G, de Juan V, Mayo-Iscar A, Martin R, et al. (2016) The influence of the refractive correction on the vision-related quality of life in keratoconus patients. Qual Life Res 25: 1043-1051.
7. Doughty MJ, Zaman ML (2000) Human corneal thickness and its impact on intraocular pressure measures: a review and meta-analysis approach. Surv Ophthalmol 44: 367-408.
8. Dimasi DP, Burdon KP, Craig JE (2010) The genetics of central corneal thickness. Br J Ophthalmol 94: 971-976.
9. Kanski Jack J (2006) Clinical ophthalmology, a systematic approach. In: Cornea. Corneal ectasias. Sixth edition, Elsevier, China.
10. Romero-Jiménez M, Santodomingo-Rubido J, Wolffsohn JS (2010) Keratoconus: a review. Cont Lens Anterior Eye 33: 157-166.
11. Barr JT, Wilson BS, Gordon MO, Rah MJ, Riley C, et al. (2006) Estimation of the incidence and factors predictive of corneal scarring in the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) Study. Cornea 25: 16-25.
12. Fernández Pérez J, Valero Marcos A, Martínez Peña FJ (2014) Early diagnosis of keratoconus: what difference is it making? Br J Ophthalmol 98: 1465-1466.
13. Davidson AE, Hayes S, Hardcastle AJ, Tuft SJ (2014) The pathogenesis of keratoconus. Eye (Lond) 28: 189-195.
14. Wojcik KA, Blasiak J, Szaflik J, Szaflik JP (2014) Role of biochemical factors in the pathogenesis of keratoconus. Acta Biochim Pol 61: 55-62.
15. Sarker-Nag A, Hutcheon AE, Karamichos D (2016) Mitochondrial Profile and Responses to TGF-β Ligands in Keratoconus. Curr Eye Res 41: 900-907.
16. Sahebjada S, Schache M, Richardson AJ, Snibson G, Daniell M, et al. (2014) Association of the hepatocyte growth factor gene with keratoconus in an Australian population. PLoS One 9: e84067.
17. Abu-Amero KK, Kondkar AA, Azad TA, Sultan T, Kalantan H, et al. (2014) Keratoconus is associated with increased copy number of mitochondrial DNA. Mol Vis 20: 1203-1208.
18. Saee-Rad S, Raoofian R, Mahbod M, Miraftab M, Mojarrad M, et al. (2013) 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 19:2501-2507.
19. Gordon-Shaag A, Millodot M, Shneor E, Liu Y (2015) The genetic and environmental factors for keratoconus. Biomed Res Int 2015: 795738.
20. Harrison RJ, Klouda PT, Easty DL, Manku M, Charles J, et al. (1989) Association between keratoconus and atopy. Br J Ophthalmol. 73: 816–822.
21. Rahi A, Davies P, Ruben M, Lobascher D, Menon J (1977) Keratoconus and coexisting atopic disease. Br J Ophthalmol 61: 761-764.
22. Wachtmeister L, Ingemansson SO, Möller E (1982) Atopy and HLA antigens in patients with keratoconus. Acta Ophthalmol (Copenh) 60: 113-122.
23. Kriszt A, Losonczy G, Berta A, Vereb G, Takács L (2014) Segregation analysis suggests that keratoconus is a complex non-mendelian disease. Acta Ophthalmol 92: e562-568.
24. Moschos MM, Droutsas K, Sioziou A, Dettoraki M, Gazouli M (2016) Mutational Analysis of Pre-miR-184 and hsa-mir-568 in Greek Patients With Sporadic Keratoconus. Cornea 35: 631-633.
25. Hughes AE, Bradley DT, Campbell M, Lechner J, Dash DP, et al. (2011) Mutation altering the miR-184 seed region causes familial keratoconus with cataract. Am J Hum Genet 89: 628-633.
26. Synowiec E, Wójcik KA, Czubatka A, Polakowski P, Izdebska J, et al. (2015) Lack of association between polymorphisms of the DNA base excision repair genes MUTYH and hOGG1 and keratoconus in a Polish subpopulation. Arch Med Sci 11: 1101-1110.
27. Synowiec E, Wojcik KA, Izdebska J, Blasiak J, Szaflik J, et al. (2014) Polymorphisms of the apoptosis-related FAS and FAS ligand genes in keratoconus and Fuchs endothelial corneal dystrophy. Tohoku J Exp Med 234: 17-27.
28. Wójcik KA, Synowiec E, Jiménez-García MP, Kaminska A, Polakowski P, et al. (2013) Polymorphism of the transferrin gene in eye diseases: keratoconus and Fuchs endothelial corneal dystrophy. Biomed Res Int 2013: 247438.
29. Pathak D, Nayak B, Singh M, Sharma N, Tandon R, et al. (2011) Mitochondrial complex 1 gene analysis in keratoconus. Mol Vis 17: 1514-1525.
30. Wang Y, Wei W, Zhang C, Zhang X, Liu M et al. (2016) Association of Interleukin-1 Gene Single Nucleotide Polymorphisms with Keratoconus in Chinese Han Population. Curr Eye Res 41: 630-635.
31. Mikami T, Meguro A, Teshigawara T, Takeuchi M, Uemoto R, et al. (2013) Interleukin 1 beta promoter polymorphism is associated with keratoconus in a Japanese population. Mol Vis 19: 845-851.
32. Nowak DM, Karolak JA, Kubiak J, Gut M, Pitarque JA, et al. (2013) Substitution at IL1RN and deletion at SLC4A11 segregating with phenotype in familial keratoconus. Invest Ophthalmol Vis Sci 54: 2207-2215.
33. Wang Y, Jin T, Zhang X, Wei W, Cui Y, et al. (2013) Common single nucleotide polymorphisms and keratoconus in the Han Chinese population. Ophthalmic Genet 34:160-166.
34. Guan T, Liu C, Ma Z, Ding S (2012) The point mutation and polymorphism in keratoconus candidate gene TGFBI in Chinese population. Gene 503: 137-139.

35. Nowak DM, Gajecka M (2015) Nonrandom Distribution of miRNAs Genes and Single Nucleotide Variants in Keratoconus Loci. PLoS One 10: e0132143.
36. Saravani R, Hasanian-Langroudi F, Validad MH, Yari D, Bahari G, et al. (2015) Evaluation of possible relationship between COL4A4 gene polymorphisms and risk of keratoconus. Cornea 34: 318-322.
37. Kokolakis NS, Gazouli M, Chatziralli IP, Koutsandrea C, Gatzioufas Z, et al. (2014) Polymorphism analysis of COL4A3 and COL4A4 genes in Greek patients with keratoconus. Ophthalmic Genet 35: 226-228.
38. Shetty R, Sathyanarayanamoorthy A, Ramachandra RA, Arora V, Ghosh A, et al. (2015) Attenuation of lysyl oxidase and collagen gene expression in keratoconus patient corneal epithelium corresponds to disease severity. Mol Vis 21: 12-25.
39. Chaerkady R, Shao H, Scott SG, Pandey A, Jun AS, et al. (2013) The keratoconus corneal proteome: loss of epithelial integrity and stromal degeneration. J Proteomics 87: 122-131.
40. Macé M, Galiacy SD, Erraud A, Mejía JE, Etchevers H, et al. (2011) Comparative transcriptome and network biology analyses demonstrate antiproliferative and hyperapoptotic phenotypes in human keratoconus corneas. Invest Ophthalmol Vis Sci 52: 6181-6191.
41. Karolak JA, Kulinska K, Nowak DM, Pitarque JA, Molinari A, et al. (2011) Sequence variants in COL4A1 and COL4A2 genes in Ecuadorian families with keratoconus. Mol Vis 17: 827-843.
42. Han S, Chen P, Fan Q, Khor CC, Sim X, et al. (2011) Association of variants in FRAP1 and PDGFRA with corneal curvature in Asian populations from Singapore. Hum Mol Genet 20: 3693-3698.
43. Mishra A, Yazar S, Hewitt AW, Mountain JA, Ang W, et al. (2012) Genetic variants near PDGFRA are associated with corneal curvature in Australians. Invest Ophthalmol Vis Sci 53: 7131-7136.
44. Cornes BK, Khor CC, Nongpiur ME, Xu L, Tay WT, et al (2012) Identification of four novel variants that influence central corneal thickness in multi-ethnic Asian populations. Hum Mol Genet 21: 437-445.
45. Lechner J, Porter LF, Rice A, Vitart V, Armstrong DJ, et al. (2014) Enrichment of pathogenic alleles in the brittle cornea gene, ZNF469, in keratoconus. Hum Mol Genet 23: 5527-5535.
46. Rohrbach M, Spencer HL, Porter LF, Burkitt-Wright EM, Bürer C, et al. (2013) ZNF469 frequently mutated in the brittle cornea syndrome (BCS) is a single exon gene possibly regulating the expression of several extracellular matrix components. Mol Genet Metab 109:289-95.
47. Burkitt Wright EM, Spencer HL, Daly SB, Manson FD, Zeef LA, et al. (2011) Mutations in PRDM5 in brittle cornea syndrome identify a pathway regulating extracellular matrix development and maintenance. Am J Hum Genet 88: 767-777.
48. Beneteau C, Landais E, Doco-Fenzy M, Gavazzi C, Philippe C, et al. (2011) Microtriplication of 11q24.1: a highly recognisable phenotype with short stature, distinctive facial features, keratoconus, overweight, and intellectual disability. J Med Genet 48: 635-639.
49. Viana MM, Frasson M, Leão LL, Stofanko M, Gonçalves-Dornelas H, et al. (2013) A new case of keratoconus associated with Williams-Beuren syndrome. Ophthalmic Genet 34: 174-177.
50. Droitcourt C, Touboul D, Ged C, Ezzedine K, Cario-André M, et al. (2011) A prospective study of filaggrin null mutations in keratoconus patients with or without atopic disorders. Dermatology 222: 336-341.
51. Karolak JA, Polakowski P, Szaflik J, Szaflik JP, et al. (2016) Molecular Screening of Keratoconus Susceptibility Sequence Variants in VSX1, TGFBI, DOCK9, STK24, and IPO5 Genes in Polish Patients and Novel TGFBI Variant Identification. Ophthalmic Genet 37: 37-43.
52. Dehkordi FA, Rashki A, Bagheri N, Chaleshtori MH, Memarzadeh E, et al. (2013) Study of VSX1 mutations in patients with keratoconus in southwest Iran using PCR-single-strand conformation polymorphism/ heteroduplex analysis and sequencing method. Acta Cytol 57: 646-651.
53. Moschos MM, Kokolakis N, Gazouli M, Chatziralli IP, Droutsas D, et al. (2015) Polymorphism Analysis of VSX1 and SOD1 Genes in Greek Patients with Keratoconus. Ophthalmic Genet 36: 213-217.
54. Saee-Rad S, Hashemi H, Miraftab M, Noori-Daloii MR, Chaleshtori MH, et al. (2011) Mutation analysis of VSX1 and SOD1 in Iranian patients with keratoconus. Mol Vis 17: 3128-3136.
55. Czugala M, Karolak JA, Nowak DM, Polakowski P, Pitarque J, et al. (2012) Novel mutation and three other sequence variants segregating with phenotype at keratoconus 13q32 susceptibility locus. Eur J Hum Genet 20: 389-397.
56. De Bonis P, Laborante A, Pizzicoli C, Stallone R, Barbano R, et al. (2011) Mutational screening of VSX1, SPARC, SOD1, LOX, and TIMP3 in keratoconus. Mol Vis 17: 2482-2494.
57. Sahebjada S, Schache M, Richardson AJ, Snibson G, MacGregor S, et al (2013) Evaluating the association between keratoconus and the corneal thickness genes in an independent Australian population. Invest Ophthalmol Vis Sci 54:8224-8.
58. Burdon KP, Macgregor S, Bykhovskaya Y, Javadiyan S, Li X, et al. (2011) Association of polymorphisms in the hepatocyte growth factor gene promoter with keratoconus. Invest Ophthalmol Vis Sci 52: 8514-8519.