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Review Article

Climatic droplet keratopathy: an old disease in new clothes

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ABSTRACT.

Climatic droplet keratopathy (CDK) is an acquired and potentially handicapping cornea degenerative disease that is highly prevalent in certain rural communities around the world. It predominantly affects males over their forties. It has many other names such as Bietti's band-shaped nodular dystrophy, Labrador keratopathy, spheroidal degeneration, chronic actinic keratopathy, oil droplet degeneration, elastoid degeneration and keratinoid corneal degeneration. CDK is characterized by the haziness and opalescence of the cornea's most anterior layers which go through three stages with increasing severity. Globular deposits of different sizes may be histopathologically observed under the corneal epithelium by means of light and electron microscopy. The coalescence and increased volume of these spherules may cause the disruption of Bowman's membrane and the elevation and thinning of the corneal epithelium. The exact aetiology and pathogenesis of CDK are unknown, but they are possibly multifactorial. The only treatment in CDK advanced cases is a corneal transplantation, which in different impoverished regions of the world is not an available option. Many years ago, the clinical and histological aspects of this disease were described in several articles. This review highlights new scientific evidence of the expanding knowledge on CDK's pathogenesis which will open the prospect for new therapeutic interventions.

Key words: ascorbate – environment – human – keratopathy – UV-B

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History of the Disease

The first description of a corneal disease that may have been climatic droplet keratopathy (CDK) was made by Baquis in 1898; and three decades later by Lugli in 1935. Then in 1937, CDK was found by Zanettin in fishermen from the Dahlak archipelago in the Red Sea, who described a severe form of this disease leading to blindness from corneal opacity (Zanettin 1937). Since then, many descriptions of this disease have been made in different parts of the world which share in common low humidity, constant winds and chronic exposure to high levels of ultraviolet radiation (UVR): Somalia (Fretillere et al. 1967; Falcone 1954; Bietti et al. 1955), Eritrea (Bietti et al. 1955; Rodger 1973), The Persian Gulf (Bietti et al. 1955), Tunisia (Nataf et al. 1957), Libya (Gandolfi 1962), South Africa (Etzine & Kaufmann 1964; Freedman 1973a,b), Labrador and New Foundland in Canada (Freedman 1965; Young & Finlay 1975), Iceland (Forsius et al. 1970), Siberia (Forsius 1972), United States (Fraunfelder et al. 1972; Klintworth 1972), Australia (McGuinness et al. 1972; Taylor 1980), Baffin Island (Freedman 1965), Northern Canada (Forsius & Eriksson 1973; Wyatt 1973), Guinea (English 1973), North Cameroon (Anderson & Fuglsang 1976), Seychelles Islands (Pilley 1976), Finnish archipelago (Forsius 1976), Punjab India (Singh & Singh 1978), Greenland (Norn 1978), Chad (Resnikoff 1988), Titicaca region in Peru (Forsius & Losno 1985), Ruanda (Forsius et al. 1995) and more recently in Argentina (Urrets-Zavalia et al. 2006).

As Table 1 shows, CDK has received different names due to various conditions.

Clinical features, diagnosis and treatment

CDK can be classified in three levels according to the tissue involvement and clinical aspects (Fraunfelder et al. 1972; Freedman 1973a, Urrets-Zavalia et al. 2006; Urrets-Zavalia et al. 2007): *Grade 1* is characterized by multiple tiny and tightly confluent translucent subepithelial

Table 1. Different names used for climatic droplet keratopathy.

Presumed aetiology	Geographic area	By eponymum	Clinical presentation	Nature of corneal deposits	Patient's activities
Climatic Droplet keratopathy[1,2]	Dahlak Islands Blindness[5,6]	Bietti's Nodular Dystrophy[13]	Degeneratio Corneae Sphaerularis Elaioides[14]	Corneal Colloid Degeneration[20]	Fisherman's Keratitis[28]
Chronic Actinic Keratopathy[3]	Corneal dystrophy from the Tropics[7]	Bietti's Corneal Nodular Dystrophy[11]	Oily Drops Central and Superficial Primary Degeneration[15]	Corneal Hyaline Degeneration[21]	
Band-shaped Climatologic Degeneration[4]	Tropical Dystrophy[7]		Band-shaped Nodular Dystrophy[13]	Special Type of Hyaline Degeneration[22]	
	Nodular Corneal Dystrophy from Tropical Countries Belt with Arid Soils[8]		Band-shaped Hyaline Nodular Keratopathy[16]	Corneal Hyaline Granular Degeneration[23]	
	Labrador's Keratopathy[9]		Spheroid Degeneration[17]	Keratinoid Corneal Degeneration[24]	
	Nama's Keratopathy[10]		Gelatinous Dystrophy[10]	Proteinaceous Corneal Degeneration[25]	
	Labrador's Keratitis[11]		Droplets Keratopathy[18]	Elastotic Corneal Degeneration[26]	
	Eskimos Keratopathy[22,12]		Corneal Droplets Degeneration[19]	Corneal Elastosis[27]	

References: 1: Freedman (1973a,b); 2: Gray et al. (1992); 3: Klintworth (1972); 4: Forsius (1972); 5: Zanettin (1937); 6: Rodger (1973); 7: Falcone (1954); 8: Bietti et al. (1955); 9: Freedman (1965); 10: Freedman (1973a,b); 11: Tremblay & Dube (1974); 12: English (1973); 13: Etzine & Kaufmann (1964); 14: Lugli (1935); 15: Alajmo (1953); 16: Duke-Elder & Leigh (1965); 17: Fraunfelder et al. (1972); 18: Garner et al. (1976); 19: Anderson & Fuglsang (1976); 20: Baquis (1898); 21: Sachsalber (1901); 22: Parsons (1904); 23: Kozlowski (1953); 24: Garner (1970); 25: Christensen (1973); 26: Brownstein et al. (1973); 27: Rodrigues et al. (1975); 28: Prasadrao (1961).

deposits, localized close to the temporal and/or nasal limbus, better seen with back-scattered slit-illumination and high magnification. A peri-limbal fringe of clear cornea is frequently observed (Fig. 1A and B). At this stage, visual acuity is uncompromised.

In *Grade 2,* the haziness spreads over the inferior 2/3rds of the cornea, giving a tarnished appearance (Fig. 1C and D). The fact that the superior cornea is shielded by the upper lid suggests a contributing etiological factor for chronic exposure of the cornea to UV illumination and other environmental stress factors. Visual acuity is moderate to severely affected.

Grade 3 is characterized by the presence of clusters of golden subepithelial droplets of different size, some of them 1 mm in diameter, extending is size, covering the cornea as the disease progresses (Fig. 1E and F). In advanced cases, areas of vascularized anterior stromal opacification or fibrosis may be observed. Once the central cornea is densely compromised, the severe visual loss that ensues may be definitive in these patients. In advanced stages of the disease, an important decrease in corneal sensitivity may lead to corneal trophic changes, perforation and permanent visual loss (Ormerod et al. 1994).

In addition to corneal findings, solar radiation chronically reaching the more exposed inferior part of the iris could also play a role in inducing iris depigmentation or atrophy in its anterior layers, as we have previously observed in 30% of CDK patients (Urrets-Zavalía et al. 2007) (Fig. 1E).

Clinical presentation and severity of lesions may vary significantly according to a particular region and its climate conditions. More severe forms of CDK have been described in regions with high heat and dryness, such as the Red Sea islands (Zanettin 1937; Rodger 1973), when compared with the corneal involvement described in patients from cold, regions such as Labrador and the Arctic Polar Circle (Freedman 1965; Forsius 1972).

CDK is a clinically well-defined entity, and its diagnosis is easy for an experienced ophthalmologist, even in its earliest stages, provided that a careful slit-lamp examination is performed. Differential diagnosis includes all diseases that produce a tarnished clinical appearance of the cornea. Slit-lamp illumination and high magnification reveal the typical confluent translucent subepithelial deposits. The disease is bilateral although asymmetry is frequently observed, as one eye may present with grade 1 disease and the other eye grade 2 or 3. In contrast, secondary spheroidal degeneration occurs in diseased, usually blind eyes and is unilateral. Differential diagnosis needs to be made with secondary spheroidal degeneration, gelatinous drop-like dystrophy, corneal oedema, band-shaped corneal degeneration, Salzmann's nodular degeneration, climatic stromal proteoglycan keratopathy, Vogt limbal degeneration, superficial corneal dystrophies, such as Reis-Buckler and granular dystrophy, and peripheral hypertrophic corneal degeneration (Järventausta et al. 2014).

Currently, no pharmacological treatment for CDK is available leaving corneal transplantation, the only choice of treatment for advanced stages of the disease. Yet, recurrence of CDK has been observed in grafted patients both with lamellar and penetrating keratoplasty between 3 ½ years and 7 years after surgery. In both cases, the disease began in the horizontal periphery of the graft and extending centrally. Those recurrences occurred in patients that continued to be exposed persistently to etiological factors (Al-Rajhi & Cameron 1996). Grafting, although relatively effective, is not suitable for patients living in remote and impoverishes regions of the world.

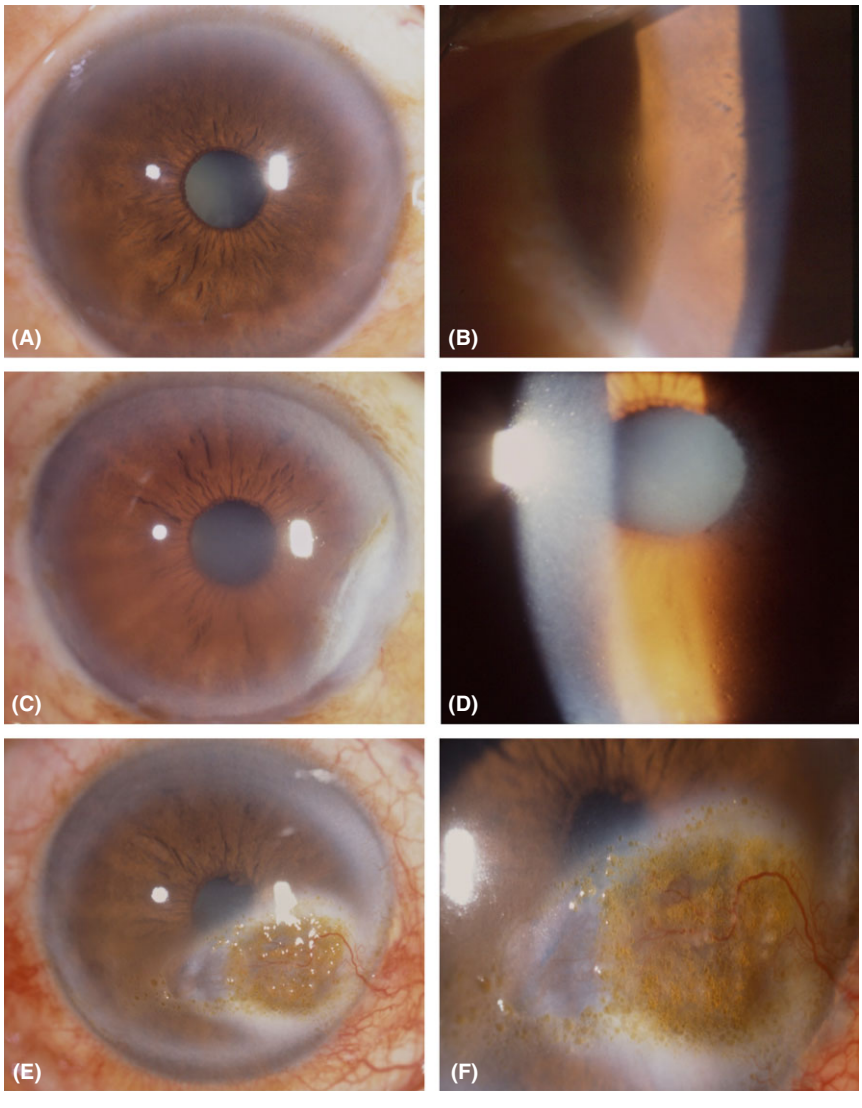

Fig. 1. Slit-lamp images of different degrees of climatic droplet keratopathy (CDK). (A) Right eye of a female rural labourer with *Grade 1* disease with peripheral nasal and temporal haziness. (B) Same eye in A, where temporal tiny droplets of different sizes are better visualized with high magnification and back-scattered slit-illumination. (C) Right eye of a male rural labourer with *Grade 2* disease; band-shaped haziness of the inferior half of the cornea is blurring iris details. (D) Central cornea of same eye in C, where tiny confluent microdroplet haziness is nicely evidenced with high magnification and back-scattered slit-illumination. (E) Right eye of a male rural labourer with *Grade 3* disease; amber-like subepithelial droplets of different sizes spread in an area of band-shaped microdroplet haziness, some of them very confluent over an area of a vascularized anterior stroma scar. (F) Same eye in E, observed with high magnification.

Recent Findings in the Pathogenesis of CDK

An important body of new information has accrued in the last years since the clinical and histological aspects of CDK were reviewed by Klintworth (Klintworth 2008).

Confocal laser scanning microscopy (CLSM)

In the last 6 years, we as well as other researchers have added new cellular level insight of this degenerative corneal disease. Although many years ago, the globular deposits in the most anterior layer of the CDK cornea were described by means of light and electron microscopy (Garner et al. 1973; Johnson & Overall 1978), more recently CDK abnormalities have been studied by us using *in vivo* confocal laser scanning microscopy (CLSM). Clinical confocal microscopy has been developed in order to overcome some of the conventional light and electron microscopy limitations such as the need of having to fix and process samples before their evaluation. In the late 1980s, the technological advances led to the development of the clinical confocal microscope which allows the detailed visualization of a patient's corneas at cellular level. Thanks to this technique, it has been possible to obtain cell images at the different cornea layers, classify them, and estimate their density (Cavanagh et al. 1990; Ruggeri & Pajaro 2002). The nerve distribution within the cornea has been elegantly studied using histopathological staining (Müller et al. 1996, 1997), but CLSM has provided a technique to examine the change in the innervation density associated with trauma and pathological conditions in the patient's cornea (Auran et al. 1995). Although corneal degenerations and dystrophies have already been studied by means of CLSM, it was not until very recently that corneal abnormalities in patients with CDK were described by us using this technique (Urrets-Zavalia et al. 2012).

CLSM images of the most relevant features at the different CDK stages can be seen in Figs 2 and 3. At its early stage (grade 1), the disease showed incipient changes with reflective punctiform or dot-like deposits in the basement membrane and Bowman's layer. A diffuse mild back-scattered reflectivity was observed in the superficial stroma, and the sub-basal nerve plexus was normal.

At its moderate and advanced stages (grades 2 and 3), an increased reflectivity of the superficial corneal epithelium and condensation of the punctuate deposits within Bowman's layer and the corneal stroma were observed. The sub-basal nerve plexus density decreased and the nerves were morphologically altered. We also observed some round hyporeflective deposits within the hyper-reflective anterior stroma that may be related to the keratinoid droplet ones. Contrary to the normal stromal nerves and branching found in grade 1, the stromal nerves presented irregular configuration and thickness in grades 2 and 3.

The initial changes do not seem to affect the sub-basal nerve plexus, but later deposit formations lead to changes in the sub-basal and stromal nerves which produce alterations that may be responsible for the corneal hyposensitivity found in these patients (Urrets-Zavalía et al. 2007). The

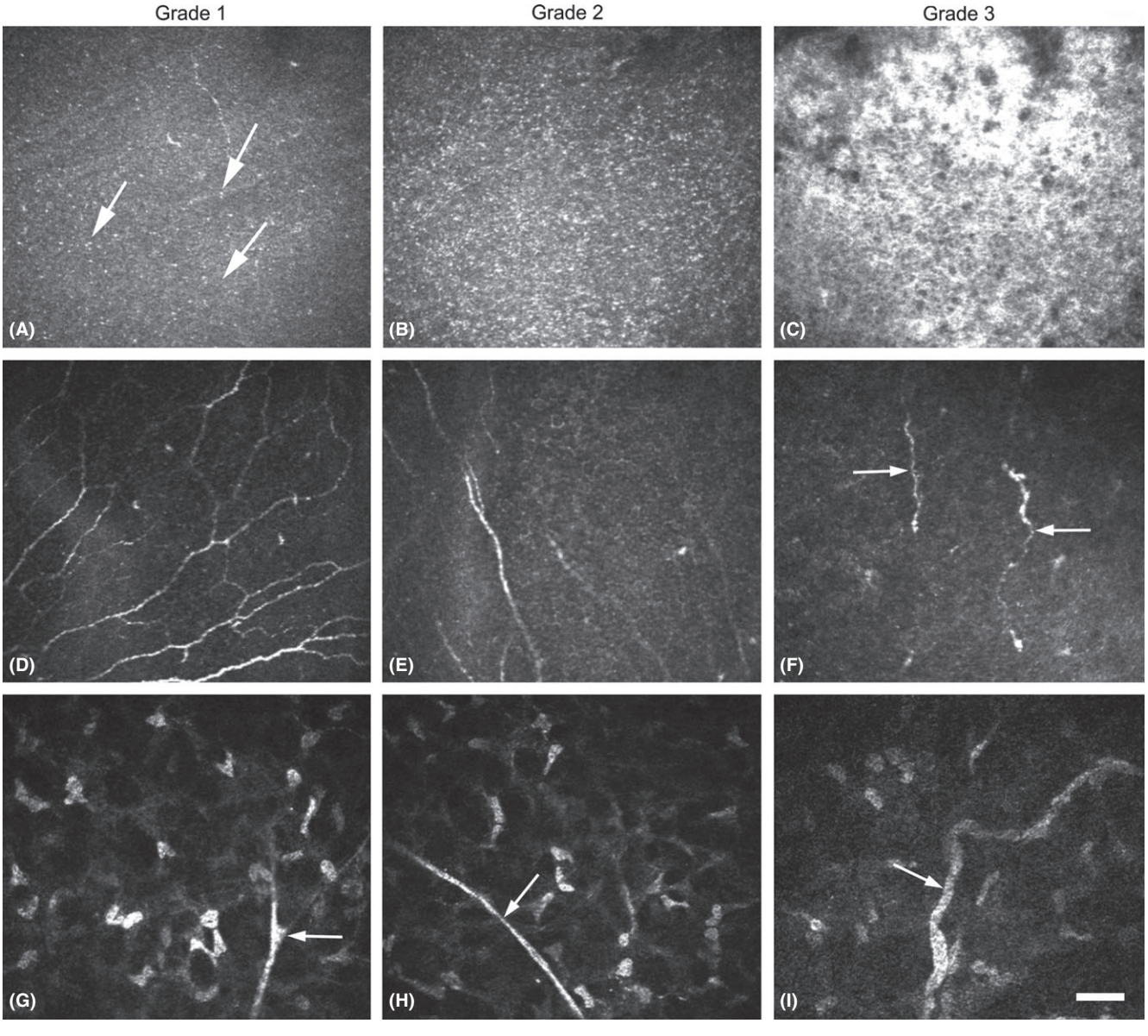

Fig. 2. *In vivo* confocal laser scanning microscopy images. Bowman's layer. (A) Grade 1: numerous dot-like hyper-reflective deposits (arrows) in the central cornea. (B) Grade 2: increased density of the deposits. (C) Grade 3: confluent hyper-reflective deposits. Sub-basal nerves. (D) Grade 1: normal appearance and density of sub-basal nerve plexus. (E) Grade 2: decrease density of nerve fibres with abrupt nerve termination. (F) Grade 3: extremely diminished nerve density and fragmented nerve fibres (arrows). Stromal nerves. (G) Grade 1: Normal nerve and branching (arrow). (H) Grade 2: Nerve with uneven thickness (I) Grade 3: Irregular configuration of nerve. (Bar = 50 μm). Modified from Eye (Urrets-Zavalia et al. 2012) with permission.

pathological consequences on nerves caused by the accumulation of extracellular material may be similar to the Corneal Lattice Dystrophy type II pathogenesis (Meretoja disease). In this disease, the accumulation of abnormal gelsolin protein leads to destruction of corneal nerves (Rosenberg et al. 2001; Mattila et al. 2014).

At advanced stages, we observed subepithelial stromal hyper-reflective plaques, activated keratocytes and peripheral vascularization. The progression of the disease was accompanied with an increment of the dendritic cells in the limbal area (87 ± 7 cells/mm^2 in grade 1, 101 ± 7 cells/mm^2 in grade 2 and 237 ± 8 cells/mm^2 in grade 3 CDK), and with a compromise of the epithelium (inclusion bodies) and the basal architecture (vacuoles) which may be secondary to fibrosis and hypo-esthesia (Urrets-Zavalía et al. 2012). The deep corneal stroma and corneal endothelium were not affected in CDK (data not shown).

New histochemical findings

As mentioned before, very little research was performed in the previous century to try to elucidate the molecular mechanisms involved in the pathogenesis of CDK. Despite of the fact that some of its constituents were identified in the droplets (Tabbara 1986; Duhaiman et al. 1988), their precise composition remains unknown. Few years ago, Fujii et al. prepared and characterized a polyclonal antibody against D-beta-Asp-containing peptides (Fujii et al. 2000) which was used to demonstrate the accumulation of D-beta-Asp-proteins contained in the skin and in various parts of the eyes including the lens, sclera, ciliary epithelium, internal limiting membrane, lamina cribrosa and drusens

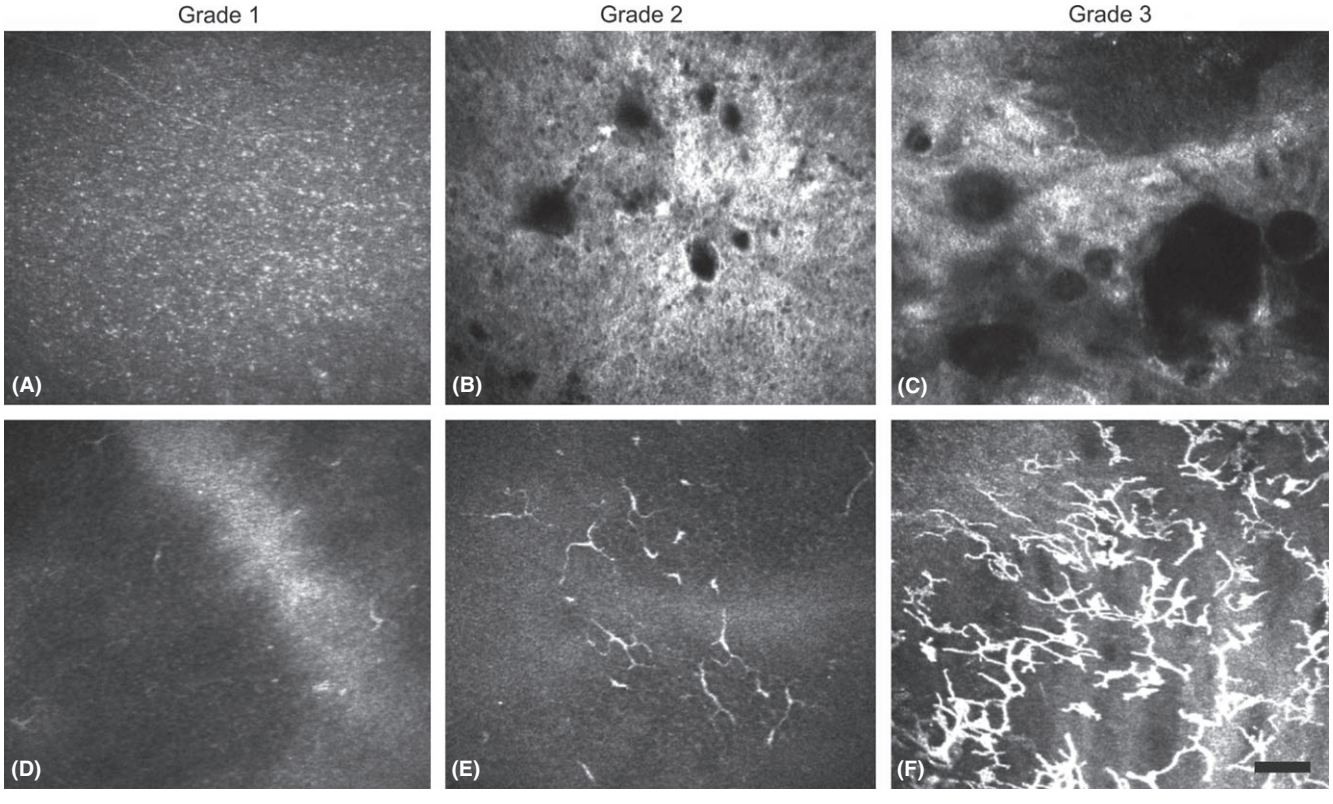

Fig. 3. *In vivo* confocal laser scanning microscopy images. Deposits at the peripheral cornea (A) Grade 1: Reflective punctiform or dot-like deposits (B) Grade 2: Hyper-reflective punctate and homogeneous round globular non-reflective deposits (C) Grade 3: Condensation of punctiform deposits; large globular non-reflective deposits. Dendritic cells at the peripheral cornea and limbus. (D) Grade 1: low density of DC, (E) *G*rade 2: moderate density of DC (F) *G*rade 3: high density of DC. (Bar = 50 μm). Adapted from Eye (Urrets-Zavalia et al. 2012) with permission.

(Kaji et al. 2007b). In 2010, the same investigators reported abnormal accumulation of D-beta-Asp-proteins in three CDK corneal specimens and in the pinguecula, but not in corneas with bullous keratopathy, interstitial keratitis or normal corneas (Kaji et al. 2010).

A previous study performed by the same group showed that the amorphous materials found in the same three CDK patients were aggregated proteins that contained advanced glycation end products (AGEs), including N-(carboxy) methyl-L-lysine, pyrraline, pentosidine and imidazolone (Kaji et al. 2007a). AGEs take a long time to form because enzymes are not involved in their formations. As all these structural changes lead to the accumulation of altered proteins, the authors proposed that the formation and accumulation of AGEs and D-beta-Asp-containing proteins underlie CDK pathogenesis.

CDK tear biomarkers

The tear fluid contains proteins/peptides, electrolytes, lipids and small molecule metabolites. Its sources include the main and accessory lacrimal glands, ocular surface epithelial cells, meibomian glands, goblet cells and an ultra filtrate of blood. Glycoproteins in tears are known from earlier studies which have generally used SDS-PAGE or immune-based techniques, but only a few have been identified (Schultz et al. 2002; Zhou & Beuerman 2012). The importance of protein glycosylation is the pattern which then reflects the ability to modify cell function especially of immune cells. However, glycosylation also stabilizes a protein in an otherwise hydrolytic environment which is the case for the tears. Using modern methods of mass spectrometry, it has been shown that tears contain over 1500 identifiable proteins (Kaji et al. 2007a). The tear fluid variations associated with disease processes are an ideal source for discovering biomarkers which lead to a better understanding of the underlying pathology (Zhou et al. 2012).

As CDK is an ocular surface disease, analysing the tear protein profile has been very informative to understand its pathology. We have analysed tears obtained from CDK patients and controls using glycopeptide capture and isobaric tags for relative and absolute quantitation (iTRAQ) (Lei et al. 2009). This study identified 43 unique N-glycoproteins, 19 of which had not been previously reported in tear fluid. The quantitative analysis in patients' tears showed that haptoglobin, polymeric immunoglobulin receptor, immunoglobulin J chain and an uncharacterized protein DKFZp686M08189 were significantly increased in their levels of N-glycosylation. In contrast, lacritin was found to be decreased in the level of N-glycosylation in CDK samples. However, the overall levels of these proteins in the tears did not change in CDK; rather, the change was in the shift from the glycosylated to the non-glycosylated forms. Our results suggest that enzymatic glycosylation may also be involved in the CDK deposit formation because increased N-glycosylation levels of some serum-origin proteins were observed in tears from CDK patients (Lei et al. 2009). The exact role of these findings remains notably elusive and cannot point to a specific pathway leading to the development of CDK. Although it was not

determined, the decrease in glycosylation may destabilize lacritin resulting in decrease bioavailability for the cornea in this disease. N-linked glycoproteins are in low abundance in biological fluids (Berman 1991), but they have additional importance as they represent a large component of the secreted proteome with a role in extra-cellular signalling (Roth 2002). As pointed out, these patterns of glycosylation are not unique but in each instance their role must be found by further studies.

Participation of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) in CDK

We have investigated the biological features of matrix metalloproteinases (MMPs) and their inhibitors TIMPs in patients with CDK, as these molecules control the degradation of the corneal epithelium and stroma. Our preliminary studies showed enhanced MMP-2 and MMP-9 levels and a decreased expression of TIMP-1 in CDK patients' tears (Holopainen et al. 2011). The source of these enzymes remained obscure, and therefore, we undertook a more detailed study using immunohistochemistry as well as corneal epithelial cell cultures to obtain further insight of these enzymes role in the development of CDK. We found that MMPs were highly upregulated in CDK patients' corneas compared to the control groups' ones. We also investigated the effect of ultraviolet B (UV-B)-irradiation in the production of MMPs and cytokines using an *in vitro* cellular model of immortalized human corneal epithelial cells (HCE). Exposure of HCE cells to UV-B irradiation significantly increased MMP and pro-inflammatory cytokine secretion. The pro-inflammatory pattern of cytokine release, especially IL-1β and IL-8 as well as TNF-α, was also observed in tears from CDK patients (Holopainen et al. 2012). This fact may be somewhat self-evident, but the novelty here is that the CDK epithelium may be the triggering factor in its pathogenesis. Accordingly, the epithelium could be the pivot point in other chronic corneal diseases such as dry eye, allergic keratoconjunctivitis and chronic ocular surface inflammation. Our data suggest that CDK pathogenesis is driven by a significant inflammatory response and that a deficient antiproteolytic shield is likely to render the cornea vulnerable to enhanced MMPs (Fig. 4). Therefore, non-specific MMPs inhibitors and inhibitors of other proteinases could be used to prevent CDK progression.

Possible protective role of vitamin C in CDK genesis

Vitamin C/ascorbic acid (AA) is synthesized by different pathways in the animal and vegetable kingdoms. In the case of fish, amphibians, reptiles and older bird orders, this vitamin is produced by the kidney, while in more recent bird orders and mammals, it is produced by the liver. Bats, guinea-pigs and anthropoid primates including humans, have lost the ability to synthesize vitamin C due to the absence of L-gulono γ-lactone oxidase (GLO), which converts L-gulonolactone to L-ascorbic acid as a result of mutations on its gene. For this reason, vitamin C must be incorporated in these animals' diets. After the introduction of the idea that ultraviolet radiation (UVR) is a potential source of eye tissue damage (protein alteration, DNA fragmentation, generation of free radicals, lipid peroxidation, etc.), numerous studies have reported the correlation between AA concentration in the cornea and its protective role against UVR exposure (Serra et al. 2014).

In human beings, it has been reported that corneal haze following photorefractive keratectomy has only occurred in Norway when the sun is visible 24 hours/day (Stojanovic & Nitter 2001).

In a non-randomized retrospective study, the same group of scientists indicated that the pre- and postoperative vitamin C supplementation can reduce the incidence of corneal haze, although they stated that the conclusions still need to be verified by future prospective randomized control trials (Stojanovic et al. 2003).

Our group has shown that CDK in the Argentinean Patagonia is a rural disease that affects individuals who generally work as sheep herders in the open air most of the day all year round, in a windy region characterized by dry and sandy soil sparsely covered by small bushes. Due to the fact that the main food source for its inhabitants is almost exclusively lamb, they have abnormally low ascorbic blood levels and consequently, lifetime partial AA nutritional deficiency (Cafaro et al. 2006).

It is important to remark that we have not found CDK in Jujuy and Santiago del Estero, other regions in Argentina with similar weather conditions and inhabitant labour activities, possibly because people in these regions tend to protect their eyes from UVR and have more varied diets with appropriate AA levels (manuscript in preparation). In addition, we have also found that apart from having moderate corneal abrasions, sheep from the same Patagonian region do not suffer from any subepithelial degenerative cornea disorders, even though they are

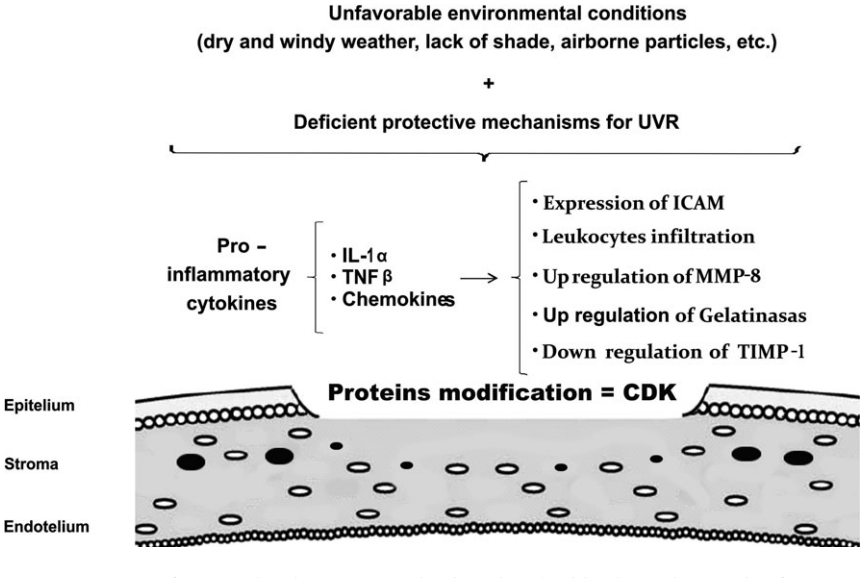

Fig. 4. Sequences of events showing some mechanisms involved in the pathogenesis of CDK.

exposed to the same environmental conditions as CDK patients (Cafaro et al. 2010). It is worthy to note that sheep, unlike men, are able to synthesize AA from the grass they eat.

Our hypothesis for CDK genesis is that individuals with chronic corneal exposure to multiple unfavourable environmental conditions (e.g. excessive UV-B exposure, lack of vegetation/shade, dry/windy climate, particle bombardment, AA partial nutritional deficiency, lack of eye protection, genetic factors, etc.) would develop inflammatory processes and oxidative stress leading to progressive degradation and accumulation of proteinaceous material in Bowman's layer and superficial stroma.

Stress response induced inflammation

Every cell has sensors that detect different forms of stress (UV-light or oxidative stress) and promote cellular adaptation (Robciuc et al. 2012). Molecular chaperones are the only sensor system in cells with the ability to repair damaged proteins (Kästle & Grune 2012). Chronic oxidative stress evokes the formation of detrimental protein aggregates. Heat-shock proteins (Hsps) functioning as molecular chaperones primarily attempt to restore proteins to their original folding state, keep their function and prevent a detrimental aggregation process. If Hsps response is exceeded, the abnormal proteins are labelled with a small polypeptide ubiquitin (Ub) that transfers the protein complex to the proteasomal protein degradation system for its elimination. The proteasome is a multicatalytic intracellular proteolytic enzyme complex that recognizes and selectively degrades oxidatively damaged and ubiquitinated proteins. Our previous proteomic analyses have interestingly shown that both Hsps and Ub are present in CDK droplets (Menegay et al. 2008). This discovery reveals that Hsps sensors in the cornea cells respond to elevated environmental or metabolic stress but due to an impaired proteasomal function, this leads to the accumulation of Ub conjugates and protein aggregates (K. Kaarniranta unpublished data). Although ECM molecules are major components in partially purified CDK droplets, Hsps and Ub provide evidence for disturbed proteostasis in CDK pathology. Moreover, recent findings reveal that protein aggregation may induce cytokine production and tissue degradation as it occurs in CDK (Hafner-Bratkovič et al. 2012).

Unmet Needs and Future Directions

Although we have made some significant advances towards the understanding of CDK pathogenesis over the past few years, we still lack detail of molecular events taking place during CDK onset and progression. With this information, it will be possible to search for new treatments. That is why we are currently working in the following areas:

Development of an animal model

Our investigations have revealed that guinea-pigs' corneas are histologically similar to humans' (Cafaro et al. 2009). As these animals' corneas lack from L-gulonolactone oxidase, their diet – as well as to humans' – has to contain AA, which will later concentrate in the corneal epithelium. For that reason, we have used four groups of guinea-pigs (A, B, C and D) to try to reproduce this human disease. In a recent preliminary study, we have investigated the effect of ascorbate deficiency and/or UV-B exposure on their corneal stromal ultrastructure. For almost 4 months, groups A and C were fed on an ascorbate-rich diet while the other two groups on an ascorbate-deficient diet. At the same time, groups C and D experienced chronic UVB exposure (0.12 J/cm^2 for 40 min/day) for the last 3 months. After the treatment, the guinea-pigs were euthanized. Transmission electron microscopy was carried out as well as a small-angle X-ray scattering to obtain the collagen fibril separation distance and the fibril diameter. Although there was no evidence of UV-B-induced collagen aggregation, collagen fibrils were found to be more closely packed in animals fed on an ascorbate-deficient diet and/or exposed to UVB. They also presented more pronounced changes in their corneal ultra structure (Hayes et al. 2011). This finding suggests that AA plays a vital role in the stroma organization and in the protection against UV-B harmful effects.

Molecular studies of purified droplets

The molecular identification of the substances forming the deposits could be valuable to understand CDK pathogenesis and provide insights into its treatment and prevention. Though the use of mass spectrometry we have found 105 proteins containing mainly secreted extracellular matrix (ECM) proteins and plasma proteins in corneas affected by CDK. The most frequent pathway for which the proteins have been identified were cell junction, focal adhesion and regulation of cytoskeleton, in addition to energy metabolism associated proteins, suggesting that several of them could play a role in deposit formation (Menegay et al. 2008).

Further protein identification in purified droplets dissected from CDK specimens will be essential to identify the proteins deposited within the droplets. Corneal tissue enriched with droplets will be obtained from CDK affected human corneas by laser capture micro-dissection (LCMD), and they will be further analysed by means of mass spectrometric techniques.

Genetic studies

We have recently shown a lack of correlation between genetic ancestry (as represented by haploid genetic systems) and the incidence of CDK in Argentina (Schurr et al. 2013). The mtDNA appears to play less of a role in CDK expression than in other complex diseases linked to bio-energetic processes. However, further analysis of the mtDNA genome sequence and other genes involved in the corneal function may reveal the role mitochondria would play in CDK's expression.

As ALDH3A1 gene encodes a protein that protects the mammalian cornea from harmful UVR, we have decided to explore its role in CDK. Genetic variation at this locus in the population who suffers from CDK is being examined through the sequencing of the entire ALDH3A1 gene, including its introns and regulatory regions in an effort to identify CDK associated variants.

The positive cross-talk between inflammatory cytokines and MMPs is well known, and our data suggest that CDK pathology is associated to a significant inflammatory response and that corneal epithelial cells play impor-

tant roles in this process. On the other hand, the sustained inflammatory condition favours the MMPs-TIMPs deregulation and its degrading consequences with a constant need of tissue remodelling. Certain polymorphisms in the TNF-α and TGF-β1 genes are related to level variations in cytokine production, some of which have been associated with inflammatory human diseases (Wang et al. 2011; Radwan et al. 2012; Tang et al. 2012). For this reason, we are also trying to assess whether TNF-α and TGF-β1 polymorphisms are among other factors that influence the development of CDK.

Conclusions

CDK is a degenerative and potentially handicapping human corneal disease suffered by individuals who work outdoors, characterized by accumulation of subepithelial protein deposits and progressive corneal opacity. The clinical and histological aspects of this disease were described many years ago, but it was not until the last decade that new contributions about molecular mechanisms involve in this disease was published.

In this review, we summarized new findings about CDK such as the subbasal and stromal nerves abnormalities, the formation and accumulation of AGEs and D-beta-Asp-containing proteins in the affected areas, an abnormal tear proteomic profile, the participation of a hypersensitivity reaction in the cornea of patient involving pro-inflammatory components of the innate immunity and a lifetime partial AA nutritional deficiency.

All these results let us to hypothesize that CDK arises in the cornea of people chronically exposed to unfavourable environmental conditions (UV-B, lack of vegetation/shade, dry climate with windy conditions, particle bombardment, partial nutritional deficiency AA, lack of eye protection and genetic factors). Oxidative stress and inflammatory processes lead to progressive degradation and accumulation of proteinaceous material in Bowman's membrane and the superficial stroma.

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